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Synthesis and *In vitro* Anti-HIV-1 Activity of Electrochemically Reduced Praseodymium Salts of 12-tungstophosphate Acid

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Abstract: A praseodymium salt of 12-tungstophosphate acid, $Pr[PW_{12}O_{40}]$, was synthesized using ion exchange and double decomposition method. Then its 2e and 4e electrochemically reduced heteropoly blues, $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ and $PrH_4[PW^{VI}_8W^V_4O_{40}]$, were synthesized using control-potential electrolysis reduction method. These compounds were characterized with FT-IR, UV-vis, polarography, ESR and XPS. The α-Keggin type structure was kept in the heteropoly blues $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ and $PrH_4[PW^{VI}_8W^V_4O_{40}]$ but the electronic density of heteropoly anion increased after electrochemically reduction. Heteropoly blues $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ and $PrH_4[PW^{VI}_8W^V_4O_{40}]$ showed relatively high *in vitro* anti-HIV-1 activity and relatively low cytotoxicity on MT-4 cells compared to some other anti-HIV-1 compounds. Because heteropoly blues possess higher negative electronic charges than their non-reduced parents, they strongly interact with HIV-1, MT-4 cells and macrophages and effectively suppress fusion, infection and proliferation of HIV-1.

Key words: Heteropoly blue, praseodymium salt, anti-HIV-1 activity, cytotoxicity on MT-4 cells

Introduction

Polyoxometalate (including heteropoly oxometalate and isopoly oxometalate) is a large class of inorganic transition metal oxygen anion clusters (Pope, 1983). Because a heteropoly anion consists of one central atom (P, Si, Ge, etc.) surrounded by some coordinate atoms (W, Mo, V, etc.), hereropoly anions usually possess cage-like molecular structures with some unique properties. In general, the coordinate atoms in heteropoly oxometalates exhibit in the highest valence (the d^0 species: W^{VI}, Mo^{VI}, V^V, etc.). The coordinate atoms in some heteropoly anions can be reduced to form the electrochemically reduced species. Because most of the electrochemically reduced species look blue no matter what colors their non-reduced parents are, these electrochemically reduced species are also called as heteropoly blues. Heteropoly blues usually retain the general structures of their non-reduced parents, but have some special properties that their oxidized parents do not have (Wang *et al.*, 1992, 1996; Yuan *et al.*, 2003). Thus heteropoly blue is a large potentially important group of mixed-valence heteropoly oxometalates.

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Heteropoly oxometalates are remarkable for their molecular and electronic structural diversity and they are promising in catalysis and material filed (Liu *et al.*, 2000, 1996). In recent years, the application of heteropoly oxometalates in medicine filed has made great progress (Rhule *et al.*, 1998; Liu *et al.*, 2005). As early as the acquired immune deficiency syndrome (AIDS) caused by human immunodeficiency virus type 1 (HIV-1) was discovered, heteropoly oxometalates have been studied as anti-AIDS inorganic compounds (Dagani, 1986). $(NH_4)_{17}Na[NaSb_9W_{21}O_{86}]•14H_2O$ (HPA-23) was the first heteropoly oxometalates applied to clinical trials for the AIDS patients (Dormont *et al.*, 1985; Balzarini *et al.*, 1986). But owing to its higher toxicity, HPA-23 could not be put on the market as a medicine (Moskovitz *et al.*, 1988). However, after Keggin structure heteropoly oxometalates (especially $K_7[PW_{10}Ti_2O_{40}]•6H_2O$ (PM-19)) were found to possess low toxicity and high anti-HIV activity from 1990s (Inouye *et al.*, 1990; Hill *et al.*, 1990), the study on the anti-HIV effect over heteropoly oxometalates becomes a popular research field again. The counter cations also influence the anti-virus activity of heteropoly oxometalates. We have reported that rare earth salts (especially Pr salt) of $[PW_{10}Ti_2O_{40}]^{7-}$ possess higher *in vitro* anti-HIV-1 activity than $K_7[PW_{10}Ti_2O_{40}]•6H_2O$ (PM-19) (Liu *et al.*, 1996). Moreover, $(iso-PrNH_3)_6H[PW_{10}Ti_2O_{38}(O_2)_2]•9H_2O$ (PM-523) has also been reported as a broad spectrum antimyxovirus agent (Shigeta *et al.*, 1996). These results indicate that Pr ion is a good counter cation for heteropoly oxometalates to improve their anti-virus activity.

Although a lot of heteropoly oxometalates had been investigated for their anti-HIV activity (Rhule *et al.*, 1998; Inouye *et al.*, 1990; Hill *et al.*, 1990; Liu *et al.*, 1996; Shigeta *et al.*, 1996; Barnard *et al.*, 1997; Shigeta *et al.*, 2003; Weeks *et al.*, 1992; Kim *et al.*, 1994), the anti-HIV activity of heteropoly blues was not carefully investigated till now except for few reports from our group in Chinese (Liu *et al.*, 2004).

Based on the experiences of anti-HIV research from one author (Y. Zeng) (Zeng *et al.*, 1984; Chen *et al.*, 2002) and the knowledge of heteropoly blues from one author (E. Wang) (Wang *et al.*, 1992, 1996; Yuan *et al.*, 2003), we synthesized heteropoly blues $PrH_2[PW^{VI}_{10}W^{V}_2O_{40}]$ and $PrH_4[PW^{VI}_8W^{V}_4O_{40}]$ in the first time and investigated their in vitro anti-HIV-1 activity in the present study.

Materials and Methods

Materials

The cooperation research for investigating *in vitro* anti-HIV-1 activity over electrochemically reduced heteropoly blues was started in 2002. The synthesis of heteropoly compounds was conducted in Institute of Polyoxometalate Chemistry, Department of Chemistry, Northeast Normal University (Changchun, China). The anti-HIV-1 experiments were performed in Department of Tumor Virus and HIV, Institute of Virology, Chinese Academy of Preventive Medicine (Beijing, China). HIV-1 was kindly provided by Prof. L. Montagnier, Institut Pasteur (Paris, France). 3'-azido-3'-deoxythymine (AZT) and Dextran surface (DS) were obtained from Tsukuba Laboratories, GlaxoSmithKline Co. (Tsukuba, Japan).

$\alpha-H_3[PW_{12}O_{40}]$ was prepared according to the method in literature (Rocchiccioli-Deltcheff *et al.*, 1983), its structure was confirmed by ^{31}P NMR and polarogram.

$Pr[PW_{12}O_{40}]$, a praseodymium salt of $\alpha-H_3[PW_{12}O_{40}]$, was prepared using an ion exchange and double decomposition method (Wang *et al.*, 1996; Tsigdinos, 1974). A portion of 1.0 g of $\alpha-H_3[PW_{12}O_{40}]$ was solved in 50 mL water and then a calculated amount of $Pr_2(CO_3)_3$ powder was

added to the solution with strong stirring. After the CO_2 gas had been eradiated, the filtrate was obtained by filtering off a small amount of solid by-product. Then, the filtrate was put in a dry box for five days to obtain the product (0.9 g). Element analyses and TG/DTA proved that the chemical composition of the product was $Pr[PW_{12}O_{40}] \cdot 18H_2O$.

Heteropoly blues, $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ and $PrH_4[PW^{VI}_8W^V_4O_{40}]$, were prepared using a control-potential electrolysis reduction method (Wang *et al.*, 1996). A solution of 1 g of $Pr[PW_{12}O_{40}] \cdot 18H_2O$ in 100 mL 0.1 M HCl solution, was placed in an electrochemical cell and reduced to a degree corresponding to the transfer of two or four electrons into per Keggin unit ($E_{control}$ = -0.40 V (2e), -0.65 V (4e) (vs. SCE)). The reduction extent was indicated by a coulometer. The cathode liquid was stirred with nitrogen stream to purge oxygen. After the electrochemical reduction, the solution was set in a vacuum desiccator. The blue crystal appeared one week later and then the product was recrystallized twice with 0.1 M HCl. The composition and the number of crystal water in the final products were analyzed with elemental analyses and TG-DTA. The chemical compositions of two electrons and four electrons reduced heteropoly blues were $PrH_2[PW_{12}O_{40}] \cdot 20H_2O$ and $PrH_2[PW_{12}O_{40}] \cdot 20H_2O$, respectively.

$K_7[PW_{10}Ti_2O_{40}] \cdot 6H_2O$ (PM-19) and $(NH_4)_{17}Na[NaSb_9W_{21}O_{86}] \cdot 14H_2O$ (HPA-23) were synthesized using the methods in the literatures (Domaille *et al.*, 1983; Fischer *et al.*, 1976; Michelon *et al.*, 1980). Both of them were confirmed by FT-IR spectra and element analyses.

Instrumentation

Inductively Coupled Plasma (ICP) analyses were carried out using a Thermo Jarrell Ash IRIS/AP instrument. TG/DTA were carried out using a Shimadzu TGA-50 instrument. FT-IR spectra were recorded on a JASCO FT/IR 7000 spectrometer using KBr pellets under atmospheric conditions. UV-vis spectra were recorded on a Beckman-DU70 spectrometer in aqueous solutions. Polarograms were recorded on a Sargernt Model XV instrument using a dropping mercury electrode and standard H cell with a saturated calomel electrode. ESR spectra were recorded on a JES-FE-3AS spectrometer. XPS spectra were collected at a chamber pressure of 10^{-9} Torr using a Phi-5500 ESCA spectrometer employing Mg K_α radiation (1253.6 eV)

Measurement of Anti-HIV-1 Activity

The MT-4 cell lines were maintained in RPMI-1640 medium supplemented with 10% fetal calf serum, 100 μg mL^{-1} penicillin and 100 μg mL^{-1} streptomycin.

The evaluation of anti-HIV activity for every compound was based on a MTT method as reported in the literature (Pauwels *et al.*, 1988). The living cells can reduce the yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to a blue formazan but the dead cells do not possess this capability. Thus the number of living cells could be calculated by the change of optical density. MT-4 cells were infected with HIV-1 at a multiplicity of infection (moi) of 0.01 at 37°C for 1 h. Then the inflected MT-4 cells were dispensed into wells of 96-well microtiter trays and incubated with individual test compounds in a total volume of 200 μL at 37°C for 5 days. The optical density of the solution was then read on a Dynatech MR700 Microelisa reader, using a test wavelength of 540 nm and a reference wavelength of 690 nm. The percentage protection was calculated using the following formula:

$$[(OD_T)V-(OD_C)V]/[(OD_C)M-(OD_C)V] \times 100(\%),$$

where $(OD_T)V$, $(OD_C)V$ and $(OD_C)M$ indicated the absorbance of the test sample (treated by virus and compound), the virus infected sample (no compound) and the mock infected control sample (no virus and no compound), respectively. The median effective concentration (E_{50}) was expressed as the concentration that achieved 50% protection of virus infected cells from virus-induced destruction.

The cytotoxicity of every compound on non-infected MT-4 cells was examined by the MTT method in parallel with the anti-HIV-1 experiment. The number of living cells was examined after 5 days incubation in the presence of different concentrations of every compound without HIV infection. The median cytotoxic concentration (CC_{50}) was determined from the dose-response curve.

In order to check the reliability of the data obtained form MTT method, the cytotoxicity of every heteropoly compound was also evaluated by a Cytopathic Effect (CPE) inhibition method (Sidwell *et al.*, 1971). MT-4 cells were dispensed into wells of 96-well microtiter trays at various concentrations of heteropoly oxometalates. After incubated with growth medium at 37°C for 5 days, the complete viral CPE was observed by light microscopy. Morphological changes due to the compounds cytotoxicity were graded on a scale of 1-4: grade 1 (-) was defined as no changed MT-4 cells; grade 2 (+) was defined that the percentage of changed cells was less than 25%; grade 3 (++) was defined that the percentage of changed cells was more than 25% but less than 75%; grade 4 (+++) was defined that the percentage of changed cells was more than 75%.

The Selective Index (SI) that indicates the anti-HIV-1 activity over every compound was calculated corresponding to the ratio CC_{50}/EC_{50}.

Results and Discussion

Characterization of Heteropoly Blues

Keggin type is one of the most popular structures for heteropoly anions. As shown in Fig. 1a Keggin structure heteropoly anion consists of a central XO_4 tetrahedron (such as PO_4) surrounded by twelve MO_6 octahedra (such as WO_6). The twelve MO_6 octahedra comprise four groups of three edge-shared octhedra, the M_3O_{13} triplet, which have a common oxygen vertex connected to the central XO_4 tetrahedron. The oxygen atoms in this structure fall into four classes of
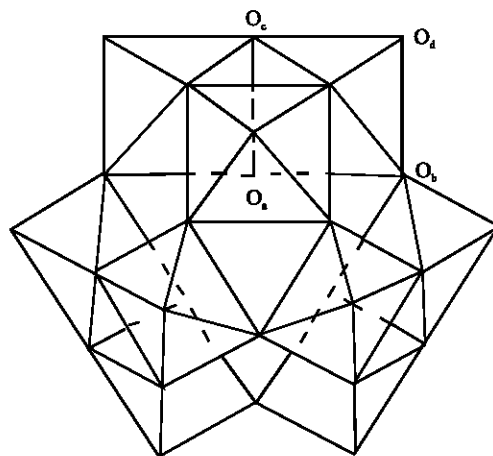

Fig. 1: The structure of Keggin type heteropoly anion

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Table 1: Peaks and their assignments in FT-IR spectra of heteropoloy compounds[a]

Compound	Vibration frequency (cm^{-1})			
	$P-O_a$	$W-O_d$	$W-O_b-W$	$W-O_c-W$
$Pr[PW_{12}O_{40}]$	1080	978	892	796
$PRH_2[PW^{VI}_{10}W^V_2O_{40}]$	1082	980	896	799
$PRH_4[PW^{VI}_8W^V_4O_{40}]$	1084	983	899	801

[a] Measured at room temperature using Kbr pellet method

Table 2: Bands and their assignments in UV-vis spectra of heteropoly compounds[a]

Compound	Band position (nm)[b]		
	$O_d \rightarrow W$	$O_{bc} \rightarrow W$	IVCT
$Pr[PW_{12}O_{40}]$	207 (380)	263 (440)	-
$PRH_2[PW^{VI}_{10}W^V_2O_{40}]$	205 (420)	261 (470)	740 (1670)
$PRH_4[PW^{VI}_8W^V_4O_{40}]$	206 (450)	260 (510)	739 (2320)

[a] Measured at room temperature in aqueous solutions, b) Molar extinction eoefficients listed in parenthesis

symmetry-equivalent oxygens: $X-O_a-(M)_3$; $M-O_b-M$, connecting two M_3O_{13} units by corner sharing; $M-O_c-M$, connecting two M_3O_{13} units by edge sharing; and $M = O_d$ (Pope, 1983). Among the heteropoly compounds used in this study, the heteropoly anions in $\alpha-H_3[PW_{12}O_{40}]$, $Pr[PW_{12}O_{40}]$ and $K_7[PW_{10}Ti_2O_{40}] \cdot 6H_2O$ (PM-19) are Keggin type heteropoly anions but the heteropoly anion in $(NH_4)_{17}Na[NaSb_9W_{21}O_{86}] \cdot 14H_2O$ (HPA-23) is not a Keggin type heteropoly anion.

The peaks and their assignments in IR spectra of $Pr[PW_{12}O_{40}]$, $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ and $PrH_4[PW^{VI}_8W^V_4O_{40}]$ are shown in Table 1. $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ and $PrH_4[PW^{VI}_8W^V_4O_{40}]$ showed the four characteristic vibrations for the Keggin type heteropoly anions ($v_{as}(P-O_a)$, $v_{as}(W=O_d)$, $v_{as}(W-O_b-W)$ and $v_{as}(W-O_c-W)$) with only slightly changes compared to those of $Pr[PW_{12}O_{40}]$. This indicates that the reduced heteropoly blues $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ and $PrH_4[PW^{VI}_8W^V_4O_{40}]$ still retain the Keggin structure with a little distortion. It is noteworthy that the frequencies of all characteristic vibrations shifted to high frequency regions from $Pr[PW_{12}O_{40}]$ to $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ and from $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ to $PrH_4[PW^{VI}_8W^V_4O_{40}]$. The electronic density in the heteropoly anion increased due to the induction of electrons during the reduction process, which caused the increase of the strengths of $P-O_a$, $W=O_d$, $W-O_b-W$ and $W-O_c-W$ bonds. Thus the characteristic vibration peaks of Keggin type $Pr[PW_{12}O_{40}]$ shifted to the high frequency regions in IR spectra after electrochemical reduction.

The data of UV-vis spectra of $Pr[PW_{12}O_{40}]$, $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ and $PrH_4[PW^{VI}_8W^V_4O_{40}]$ are shown in Table 2. Keggin structure heteropolytungstate anions have characteristic absorption bands at about 200 and 260 nm in UV region corresponding to charge transfers of O_d-W^{VI} and $O_{b,c}-W^{VI}$ (Pope, 1983). As shown in Table 2, both $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ and $PrH_4[PW^{VI}_8W^V_4O_{40}]$ showed these bands whose positions were quite similar to that of $Pr[PW_{12}O_{40}]$. These results are coincided with the results from IR spectra that the Keggin type structure was kept in the heteropoly blues $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ and $PrH_4[PW^{VI}_8W^V_4O_{40}]$ after electrochemical reduction.

The appearance of a strong absorption bond at about 740 nm in visible region is a signal for the formation of heteropoly blue. This is the reason why the electrochemical reduced heteropoly compounds look blue. This band can be assigned to Intervalence Charge-transfer (IVCT) from $W^V-O_b-W^{VI}$ to $W^{VI}-O_b-W^V$ according to its strength and position (Pope, 1983). $Pr[PW_{12}O_{40}]$ only showed a weak broad absorption band in visible region due to the absorption of Pr^{3+} cation (not shown in Table 2) and did not show any absorption bands at about 740 nm. On the other hand, both $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ and $PrH_4[PW^{VI}_8W^V_4O_{40}]$ showed strong a bsorption bands at about 740 nm,

Table 3: Polarograms data of various heteropoly compounds[a]

Compound	No. of waves	$E_{1/2}$ (cathodic)	No. of electrons
α-$H_3[PW_{12}O_{40}]$	1st	-0.096	1
	2nd	-0.345	1
	3rd	-0.624	2
$Pr[PW_{12}O_{40}]$	1st	-0.095	1
	2nd	-0.343	1
	3rd	-0.623	2
$PrH_2[PW^{VI}_{10}W^V_2O_{40}]$	1st	-0.101	1
	2nd	-0.640	1
	3rd	-0.640	2
$PrH_4[PW^{VI}_8W^V_4O_{40}]$	1st	-0.104	1
	2nd	-0.355	1
	3rd	-0.646	2

[a] Measured in 0.1 N HCl solution at room temperature

which indicates that heteropoly blues were formed after electrochemically reduction of $Pr[PW_{12}O_{40}]$. The band intensity of $PrH_4[PW^{VI}_8W^V_4O_{40}]$ at about 740 nm is stronger than that of $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ due to the induction of two extra electrons in $PrH_4[PW^{VI}_8W^V_4O_{40}]$.

The half wave potentials in polarograms of α-$H_3[PW_{12}O_{40}]$, $Pr[PW_{12}O_{40}]$, $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ and $PrH_4[PW^{VI}_8W^V_4O_{40}]$ are shown in Table 3. Both the wave shapes and the half wave potentials in polarograms are quire similar among α-$H_3[PW_{12}O_{40}]$, $Pr[PW_{12}O_{40}]$, $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ and $PrH_4[PW^{VI}_8W^V_4O_{40}]$. All of them showed three waves corresponding to one-electron, one-electron and two-electrons reduction processes. In general, the wave shapes and the half wave potentials in the polarograms are quite different among α-isomer, β-isomer and γ-isomer of Keggin type heteropoly anions (Biquard *et al.*, 1971). No significant changes were observed in polarograms after the process of ion exchange and double decomposition and after the process of electrochemical reduction. Therefore, The α-isomer of Keggin structure is concluded to be kept in $Pr[PW_{12}O_{40}]$, $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ and $PrH_4[PW^{VI}_8W^V_4O_{40}]$ as the starting heteropoly compound α-$H[PW_{12}O_4]$.

$Pr[PW_{12}O_{40}]$ did not show any ESR signals both at room temperature and at 77 K. As for $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ and $PrH_4[PW^{VI}_8W^V_4O_{40}]$, no ESR single could be detected at room temperature because of the strong spin-lattice relaxation resulting from both the electron delocalization and the thermal excited electron jumping. However, the ESR signals with isotropic line shapes could be observed at 77 K for both $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ and $PrH_4[PW^{VI}_8W^V_4O_{40}]$ at $g = 1.83$. Because this g value is the characteristic position of W^V ion, the electrons introduced into the heteropoly blues mainly localized in the orbit of W^V ions with little delocalization at low temperature like Mo^V ions containing heteropoly blues (Sanchez *et al.*, 1982).

The binding energy of electrons in inner shell of P_{2p} and O_{1s} changed little in the XPS spectra among $Pr[PW_{12}O_{40}]$, $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ and $PrH_4[PW^{VI}_8W^V_4O_{40}]$. This indicates the structures of three samples closely resemble with each other. The signals of $W_{4f7/2}$ in $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ and $PrH_4[PW^{VI}_8W^V_4O_{40}]$ were broad and deformed compared to that in $Pr[PW_{12}O_{40}]$. This phenomenon could be explained by the additive effect of the splitting peaks for both W^V and W^{VI} in the heteropoly blues. After decomposed the $W_{4f7/2}$ singles in $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ and $PrH_4[PW^{VI}_8W^V_4O_{40}]$, the ratios of the peak area of W^V to W^{VI} were almost 2:10 for $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ and 4:8 for $PrH_4[PW^{VI}_8W^V_4O_{40}]$, respectively. These results strongly suggest that the reduced heteropoly blues $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ has two W^V ions and $PrH_4[PW^{VI}_8W^V_4O_{40}]$ has four W^V ions in their structures as was expected.

As described in above, the results of FT-IR, UV-vis, polarography, ESR and XPS proved that the electrochemically reduced praseodymium salts of 12-tungstophosphate acid, $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ and $PrH_4[PW^{VI}_8W^V_4O_{40}]$, have been synthesized successfully in this study.

Table 4: Cytotoxicith and anti-HIV-1 activity of various compounds[a]

Compound	CC_{50} (μg mL^{-1})[b]	EC_{50} (μg mL^{-1})[c]	Si[d]
α-$H_3[PW_{12}O_{40}]$	262	7.5	35
$Pr[PW_{12}O_{40}]$	289	6.6	44
$PrH_2[PW^{VI}_{10}W^V_2O_{40}]$	318	4.8	66
$PrH_4[PW^{VI}_8W^V_4O_{40}]$	320	4.6	70
$K_7[PW_{10}Ti_2O_{40}]$ (PM-19)	280	5.5	51
$(NH_4)_{17}Na[NaSb_9W_{21}O_{86}]$ (HPA-23)	42	3.2	13
Dextran sulfate (DS)	697	10.1	69
Azido-2,3-dideoxythymidne (AZT)	2	0.002	1000

[a] Data represent mean values for three experiments. Cytotoxicity and anti- HIV-1 acitivity were measured by the MTT method. [b] CC_{50}: the median cytotoxic concentration of compound for achieving 50% protection of HIV infected MT-4 cell from virus-induced destruction. [d] SI: the ratio of CC_{50} to EC_{50}

Tabel 5: The cytotoxicity on MT-4 cell of various heteropoly oxometalates[a]

Compound	Dose (μg mL^{-1})[b]				Max_{non}[c]
	10	50	150	200	
$Pr[PW_{12}O_{40}]$	-	-	-	+	>150
$PrH_2[PW^{VI}_{10}W^V_2O_{40}]$	-	-	-	+	>150
$PrH_4[PW^{VI}_8W^V_4O_{40}]$	-	-	-	+	>150
$K_7[PW_{10}Ti_2O_{40}]$ (PM-19)	-	-	-	+	>150
$(NH_4)_{17}Na[NaSb_9W_{21}O_{86}]$ (HPA-23)	-	+	++	+++	<50

[a] Data represent mean values for three experiments. Morphological change of MT-4 cell was observed by light microscopy after 5 days incubation in medium with heteropoly oxometales. [b] -: no cell changed cells in the range from 25 to 75%; +++: percentage of changed cells >75%. [c] Max_{non}: the maximum non-cytotoxic dose

Cytotoxicity on Mt-4 Cells and In vitro Anti-hiv-1 Activity of Heteropoly Oxometalates

The cytotoxicity on MT-4 cells and *in vitro* anti-HIV-1 activity evaluated by the MTT method over various compounds are shown in Table 4. Keggin type heteropoly oxometalates showed their median cytotoxic concentration (C_{50}) at a range from 260 to 320 μg mL^{-1}. On the other hand, the non-Keggin type heteropoly oxometalates HPA-23 showed a low C_{50} value of 42 μg mL^{-1}, which indicates that HPA-23 possesses a high cytotoxicity on MT-4 cells. These results are consistent with the results reported in the literatures. α-$H_3[PW_{12}O_{40}]$ and $Pr[PW_{12}O_{40}]$ showed their SI values at 35 and 44, respectively. This suggests that Pr^{3+} ion possesses the ability to improve the anti-HIV-1 activity of $[PW_{12}O_{40}]^{3-}$ heteropoly anion. Because the SI values of $Pr[PW_{12}O_{40}]$, $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ and $PrH_4[PW^{VI}_8W^V_4O_{40}]$ are 44, 66 and 70, the anti-HIV-1 activity is in the order of $Pr[PW_{12}O_{40}]$ $<PrH_2[PW^{VI}_{10}W^V_2O_{40}]<PrH_4[PW^{VI}_8W^V_4O_{40}]$. A higher negative charges that a heteropoly anion possesses, a higher anti-HIV activity could be expected. $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ and $PrH_4[PW^{VI}_8W^V_4O_{40}]$ showed higher SI values than that of PM-19, which indicates that heteropoly blues $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ and $PrH_4[PW^{VI}_8W^V_4O_{40}]$ are effective heteropoly oxometalates for anti-HIV-1. DS showed low cytotoxicity on MT-4 cells and its SI value was similar to those of $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ and $PrH_4[PW^{VI}_8W^V_4O_{40}]$. On the other hand, AZT showed a high SI value of 1000 but its cytotoxicity was very high. Therefore, it seem that the mechanism of anti-HIV over heteropoly oxometalates is similar to the mechanism over DS but is different from the mechanism over AZT.

The cytotoxicity on MT-4 cells over various heteropoly oxometalates observed by the CPE effect using light microscopy are shown in Table 5. All Keggin type heteropoly compounds (PM-19, $Pr[PW_{12}O_{40}]$ and its reduced species) showed low cytotoxity on MT-4 cells and their maximum non-cytotoxic doses were higher than 150 μg mL^{-1}. On the other hand, the non-Keggin structure

heteropoly oxometalates HPA-23 showed high cytotoxicity on MT-cells, its maximum non-cytotoxic dose was lower than 50 µg mL^{-1}. Comparing the results in Table 4 and Table 5, the cytotoxicity of various heteropoly compounds did not show significant difference between using the MTT method and using the CPE inhibition method. This indicates that the MTT method is reliable for evaluating the cytotoxicity on MT-cells and *in vitro* anti-HIV-1 activity over various compounds.

As shown in Table 4 and 5, two heteropoly blues prepared in this study, $PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ and $PrH_4[PW^{VI}_8W^V_4O_{40}]$, showed higher *in vitro* anti-HIV-1 activity and lower cytotoxicity on MT-4 cells comparing to the reported heterpoly compound anti-HIV-1 agents. Moreover, although the anti-HIV-1 activity *in vitro* of heteropoly blues were lower than that of AZT, heteropoly blues showed much lower cytotoxicity on MT-cells comparing to AZT.

Several groups have studied the mechanism of the anti-HIV effect over heteropoly oxometalates (Take *et al.*, 1991; Inouye *et al.*, 1995; Judd *et al.*, 2001; Ni *et al.*, 1995; Witvrouw *et al.*, 2000). Although the mechanism of anti-HIV activity over heteropoly oxometalates has not been fully elucidated, two mechanisms including inhibition of binding/fusion process and inhibition of reverse transcriptase, are responsible for their antiviral activity. The anti-HIV activity over a heteropoly oxometalate is determined by many factors, such as shape, size, surface charge, species of heteropoly oxometalates and so on. From the views of all reported mechanisms, the high negative charge of heteropoly anions is one of the most important factors for improving the anti-HIV activity: high negative charge could promote the ability of heteropoly anions to block virion absorption to membrane receptors to inhibit the fusion process of HIV infection (Take *et al.*, 1991; Inouye *et al.*, 1995); high negative charge could improve the ability of heteropoly anions to bind to the cationic pocket on the "hinge" region of the flaps to cover the active site of HIV protease (Judd *et al.*, 2001); high negative charge could increase the accumulated ability in macrophages of heteropoly anions to suppress HIV proliferation (Ni *et al.*, 1995; Witvrouw *et al.*, 2000).

Heteropoly blues possess the same structure and the same composed elements as their non-reduced parent heteropoly oxometalates. The difference between heteropoly blues and their non-reduced parents is just that heteropoly blues possess higher negative charges. Therefore, it seem that the higher negative charges caused higher *in vitro* anti-HIV-1 activity of heteropoly blues.

We think heteropoly blues could provide us two ideas for anti-HIV research. One idea is preparing the heteropoly blues of an effective heteropoly oxometalate because heteropoly blues possess higher anti-HIV activity than their non-reduced parents. Another idea is studying the anti-HIV mechanism of heteropoly oxometalates using heteropoly blues and their non-reduced parents because heteropoly blues have same characters (shape, size and composed elements) as their non-reduced parents except that heteropoly blues have higher negative charges.

In conclusion, we successfully synthesized the electrochemically reduced praseodymium salts of 12-tungstophosphate acid ($PrH_2[PW^{VI}_{10}W^V_2O_{40}]$ and $PrH_4[PW$ W O $]$) for the first time. Because the prepared electrochemically reduced heteropoly blues remain the structures of their non-reduced parent heteropoly oxometalates but possess higher negative charge comparing to their non-reduced parent heteropoly oxometalates, they showed relatively high *in vitro* anti-HIV-1 activity and relatively low cytotoxicity on MT-4 cells.

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