# Structure–Activity Correlationship and Strain Specificity of Polyoxometalates in Anti-human Immunodeficiency Virus Activity

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**The anti-human immunodeficiency virus (HIV) activity of polyoxometalates of representative structural families, such as Keggin, lacunary Keggin, trivacant Keggin, Keggin sandwich, Wells-Dawson and Wells-Dawson sandwich, was determined using two strains of HIV type 1 (HIV-$1_{HTLV-IIIB}$ and HIV-$1_{SF-2H}$). The compounds were preferably selected to cover both polyoxotungstates and polyoxomolybdates in each structural family. In general, polyoxotungstates of Keggin, lacunary Keggin, trivacant Keggin, Keggin sandwich, Wells-Dawson and Wells-Dawson sandwich structures showed anti-HIV-$1_{HTLV-IIIB}$ activity, whereas most compounds not included in these structural categories were inactive. Among the compounds with a potent anti-HIV-$1_{HTLV-IIIB}$ activity, those of Keggin and its closely related structural families (lacunary Keggin, trivacant Keggin and Keggin sandwich) inhibited the cytopathogenicity and syncytium formation caused by HIV-$1_{SF-2H}$ to a much higher extent compared with HIV-$1_{HTLV-IIIB}$-related ones. The difference between the spectra of anti-HIV-$1_{HTLV-IIIB}$ activity and the specificity for HIV-$1_{SF-2H}$ might result from differential structural requirements in these functions.**

**Key words** polyoxometalate; human immunodeficiency virus; strain specificity; syncytium formation; cytopathogenicity

Since heteropolyoxotungstate HPA 23 $(NH_4)_{17}Na[NaSb_9W_{21}O_{86}]\cdot 14H_2O$ was first used in clinical trials for patients with acquired immunodeficiency syndrome (AIDS), which is caused by a retrovirus, human immunodeficiency virus (HIV),[1] a series of polyoxometalates have been tested for their ability to inhibit the growth of HIV by measuring their cytopathogenic effect (CPE) on human $CD4^+$ cells. Among them, Keggin heteropolyoxotungstates represented by PM-19 $K_7[PTi_2W_{10}O_{40}]\cdot 6H_2O$ were found to be potent inhibitors of HIV-$1_{HTLV-IIIB}$.[2]

In addition to CPE, syncytium formation was inhibited by PM-19,[2c] implying that polyoxometalates interrupt either virus adsorption to membrane receptors or penetration into target cells *via* a fusion mechanism. In fact, the direct interaction between polyoxometalates and envelope glycoprotein gp120 was demonstrated using anti-gp120 monoclonal antibody.[3]

The extent of inhibition of syncytium formation by a heteropolyoxomolybdate, PM-104 $(NH_4)_{12}H_2[Eu_4(MoO_4)(H_2O)_{16}(Mo_7O_{24})_4]\cdot 13H_2O$, was strain-dependent: the susceptibility of HIV-$1_{SF-2H}$-dependent syncytium formation was two orders of magnitude higher than that of the HIV-$1_{HTLV-IIIB}$-dependent one.[4] However, it remains to be addressed whether this strain specificity shown by PM-104 is shared by other polyoxometalates.

## MATERIALS AND METHODS

**Chemicals** PM-92 $H_4[SiW_{12}O_{40}]\cdot 24H_2O$ and PM-525 $Na_{10}[W_{12}O_{42}]\cdot nH_2O$ were purchased from commercial sources and used without further purification.

The following compounds were prepared and purified as described previously: PM-1 $K_5[BW_{12}O_{40}]\cdot 15H_2O$,[5] PM-3 $K_{27}[KAs_4W_{40}O_{140}]\cdot nH_2O$,[6] PM-5 $Na_6[NiMo_9O_{32}]\cdot nH_2O$,[7] PM-8 $(iso\text{-}PrNH_3)_6[Mo_7O_{24}]\cdot 3H_2O$,[8] PM-10 $K_7[NiV_{13}O_{38}]\cdot 16H_2O$,[9] PM-19 $K_7[PTi_2W_{10}O_{40}]\cdot 6H_2O$,[10] PM-29 $K_8[P_2CoW_{17}O_{62}]\cdot 18H_2O$,[11] PM-30 $Na_9[SiW_9O_{33}(OH)]\cdot 23H_2O$,[12] PM-33 $(NH_4)_6[Cr_2Mo_{12}O_{42}]\cdot 20H_2O$,[13] PM-43 $K_5[SiVW_{11}O_{40}]\cdot nH_2O$,[14] PM-44 $K_5[PVW_{11}O_{40}]\cdot nH_2O$ and PM-47 $K_7[BVW_{11}O_{40}]\cdot nH_2O$,[15] PM-48 $K_{13}[Eu(SiW_{11}O_{39})_2]\cdot nH_2O$,[16] PM-62 $K_7[PMo_2W_9O_{39}]\cdot 19H_2O$, PM-63 $(NH_4)_6H[PMo_{11}ZnO_{40}]\cdot 25H_2O$, PM-64 $Na_3H_6[PMo_9O_{34}]\cdot 13H_2O$, PM-66 $K_3[PMo_3W_9O_{40}]\cdot 25H_2O$ and PM-67 $K_3[PMo_9W_3O_{40}]\cdot 5H_2O$,[17] PM-65 $K_7[PW_{11}O_{39}]\cdot nH_2O$,[18] PM-73 $Na_{10}[SiW_9O_{34}]\cdot 18H_2O$,[12] PM-82 $K_5[PV_2W_{10}O_{40}]\cdot nH_2O$,[19] PM-83 $K_5[SiFe(H_2O)W_{11}O_{39}]\cdot 14H_2O$,[20] PM-501 $K_5H_2[V_{15}O_{36}(CO_3)]\cdot 16.5H_2O$,[21] PM-506 $K_7[CoW_{11}O_{38}]\cdot 17H_2O$.[22] PM-509 $K_{13}[Cu_3(NO_3)(PW_9O_{34})_2]\cdot nH_2O$,[23] PM-510 $K_8[(H_2O)MnP_2W_{17}O_{61}]\cdot nH_2O$,[11] PM-511 $(NH_4)_8[H_2Co_2W_{11}O_{40}]\cdot 20H_2O$,[24] PM-519 $(iso\text{-}Pr_2NH_2)_5[PTiW_{11}O_{39}(O_2)]\cdot 4H_2O$, PM-520 $(iso\text{-}Pr_2NH_2)_5[PTiW_{11}O_{40}]\cdot 4H_2O$ and M-523 $(iso\text{-}PrNH_3)_6[PTi_2W_{10}O_{38}(O_2)_2]\cdot H_2O$,[25] PM-526 $K_{17}[Eu(P_2W_{17}O_{61})_2]\cdot nH_2O$,[26] PM-527 $K_{5.5}H_{1.5}[SbW_6O_{24}]\cdot 6H_2O$,[27] PM-932 $(tert\text{-}BuNH_3)_4[V_4O_{12}]$.[28] PM-77 $(Me_4N)_7[PTi_2W_{10}O_{40}]\cdot nH_2O$ and PM-524 $Cs_7[PTi_2W_{10}O_{38}(O_2)_2]\cdot nH_2O$ were prepared according to the procedures for PM-19[10] and PM-523,[25] respectively, with some modifications. PM-113 $(NH_4)_{12}H_2[Nd_4(MoO_4)(H_2O)_{16}(Mo_7O_{24})_4]\cdot nH_2O$ was prepared by the method of Naruke *et al.*[29] HPA-23 was generously provided by Prof. Teze, Université Pierre et Marie Curie. PM-2 $K_{18}[KSb_9W_{21}O_{86}]\cdot nH_2O$ and PM-72 $(NH_4)_{18}[(NH_4)Sb_9W_{21}O_{86}]\cdot nH_2O$ were prepared by modifying the previous methods.[30]

**Cells and Viruses** All the cell lines were maintained in RPMI-1640 medium supplemented with 10% fetal calf serum; penicillin, 100 units/ml; and streptomycin, 100 μg/ml. MT-4 cells are highly susceptible to the CPE of HIV. MOLT-4 clone C10 cells were established in our laboratory and were used as target cells in a syncytium formation inhibition assay, as were MT-2 cells. MOLT-4

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cells chronically infected with HIV-$1_{SF-2H}$ (a variant of HIV-$1_{SF-2}$), HIV-$1_{HTLV-IIIB}$, HIV-$1_{MN}$, HIV-$1_{RF}$ and HIV-$2_{GH-1}$ were referred to as MOLT-4/HIV-$1_{SF-2H}$, MOLT-4/HIV-$1_{HTLV-IIIB}$, MOLT-4/HIV-$1_{MN}$, MOLT-4/HIV-$1_{RF}$ and MOLT-4/HIV-$2_{GH-1}$, respectively. MOLT-4 cells chronically infected with vaccinia virus carrying the individual *env* genes of diverse HIV-1 strains, including a macrophage-tropic HIV-$1_{SF-162}$, were newly established and named MOLT-4/Vac-*env*, (Inouye *et al.*, unpublished).

The stock of HIV-$1_{HTLV-IIIB}$ was obtained from the culture supernatant of MOLT-4/HIV-$1_{HTLV-IIIB}$ cells. The titers of the virus preparations determined in MT-4 cells 4 d after infection were $1.6 \times 10^4$ 50% tissue culture infectious dose ($TCID_{50}$)/ml.

**HIV-1-Induced-CPE Inhibition Assay** An anti-HIV assay based on the inhibition of virus-induced cytopathogenicity in MT-4 cells was carried out according to the previous method.[4] MT-4 cells were infected with HIV-$1_{HTLV-IIIB}$ at a multiplicity of infection (moi) of 0.01 at 37 °C for 1 h. Cells were dispensed into wells of 96-well microtiter trays and incubated with individual test compounds in a total volume of 200 $\mu$l at 37 °C for 5 d. The cell viability was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) metabolic assay.[31] The 50% effective concentration ($EC_{50}$) was defined as the concentration at which CPE was inhibited by 50%. The toxicity of each compound was evaluated in parallel with the determination of anti-HIV-1 activity. At the 50% cytotoxic concentration ($CC_{50}$), the cell viability of mock-infected MT-4 cells was half that of untreated cells.

The infectious virus particles were not recovered from the culture supernatant of MOLT-4/HIV-$1_{SF-2H}$ cells. However, severe cytopathogenicity was observed in MT-4 cells co-cultured with MOLT-4/HIV-$1_{SF-2H}$ at the ratio of 20:1 for 5 d. CPE, probably caused by the cell-to-cell transmission of HIV-$1_{SF-2H}$, was used to assess the anti-HIV-1 activity against this strain. The other procedures used were similar to those for the cell-free infection by HIV-$1_{HTLV-IIIB}$.

**Syncytium Formation Inhibition Assay** The syncytium formation inhibition assay was carried out as described previously.[4,32] Briefly, uninfected MOLT-4 clone C10 cells and HIV-infected MOLT-4 cells (MOLT-4/HIV-$1_{SF-2H}$, MOLT-4/HIV-$1_{HTLV-IIIB}$, MOLT-4/HIV-$1_{MN}$, MOLT-4/HIV-$1_{RF}$ or MOLT-4/HIV-$2_{GH-1}$) were cultured at a cell density of $3.0 \times 10^5$ cells in a total volume of 1.2 ml in a mixture of 2:1, or individually in the presence of various concentrations of test compounds at 37 °C for 20 h. Control wells received either uninfected cells, HIV-infected cells or the 2:1 mixture in the absence of test compounds at the same cell density as those containing test compounds. The number of cells was determined in a Coulter counter Model DN (Coulter Electronics, Ltd.). The definitions of fusion index (FI) and the 50% inhibitory concentration ($IC_{50}$) were described previously.[4]

In contrast, MOLT-4/Vac-*env* formed syncytia when co-cultured with MT-2 but not with MOLT-4 clone C10, irrespective of the tropism of a source HIV-1 strain. Furtheremore, the Vac-*env*-dependent syncytium formation could not be differentiated from the HIV-dependent one in terms of susceptibility to various inhibitors (Inouye *et al.*, unpublished).

**Immunofluorescence Assay** The effect of PM-19 on the absorption of anti-HIV-1 polyclonal antibodies to HIV-1-infected MOLT-4 cells was measured according to the method of Schols *et al.*[33] with some modification. Briefly, $1 \times 10^6$ cells of either MOLT-4 clone C10, MOLT-4/HIV-$1_{SF-2H}$ or MOLT-4/HIV-$1_{HTLV-IIIB}$ were washed twice with phosphate buffered saline containing 10 mM EDTA and 0.1% of $NaN_3$ (PBS-EDTA), and incubated at 37 °C for 30 min with 0.5 or 50 $\mu$g/ml PM-19 in 2 ml of growth medium. After being washed with ice-cold PBS-EDTA, the cells were left in 200 $\mu$l of PBS supplemented with 1% bovine serum albumin (BSA) [PBS-BSA(1%)] containing anti-HIV-1 polyclonal antibodies at 4 °C for 30 min and then washed twice again with PBS-EDTA. Further, the cells were incubated in 200 $\mu$l of PBS-BSA(1%) containing fluoresceinisothiocyanate (FITC)-conjugated goat anti-human IgG (H+L) antibody at 4 °C for 30 min, washed twice with PBS-EDTA, resuspended in 1 ml of 0.37% formaldehyde in PBS-EDTA and analyzed by flow cytometry.

## RESULTS

The CPE of HIV-$1_{HTLV-IIIB}$ is affected by the polyoxotungstates of Keggin, lacunary Keggin, trivacant Keggin, Keggin sandwich, Wells-Dawson and Wells-Dawson sandwich families (Table 1). The SI values range from 5 to 60. The polyoxomolybdates, PM-63, PM-108 and PM-64, and molybdic and tungstic polyoxometalates such as PM-66, PM-67 and PM-62, lack anti-HIV-1 activity, even though they are in the same structural families as the active polyoxotungstates. All the compounds which are not classified in the above described structural families, excluding PM-3, are also inactive against the CPE of HIV-$1_{HTLV-IIIB}$. Treatment with the polyoxovanadates PM-932, PM-10 and PM-501 were accompanied by a severe toxicity.

The CPE caused by the cell-to-cell transmission of HIV-$1_{SF-2H}$ is highly susceptible to the inhibitory effect of polyoxotungstates of Keggin, lacunary Keggin, trivacant Keggin and Keggin sandwich families, with the ratio of SI values for HIV-$1_{SF-2H}$ to HIV-$1_{HTLV-IIIB}$ being approximately 100 (Tables 1 and 2). In contrast, the increase in SI values was not significant in the cases of polyoxotungstates of Wells-Dawson and Wells-Dawson sandwich families. The CPE of HIV-$1_{SF-2H}$ was not affected by the compounds inactive against the CPE of HIV-$1_{HTLV-IIIB}$.

The syncytium formation catalyzed by HIV-$1_{RF}$, HIV-$1_{MN}$, HIV-$2_{GH-1}$ or Vac-*env*(HIV-$1_{SF-162}$) was affected by PM-19 to the same extent as that induced by HIV-$1_{HTLV-IIIB}$ (Table 3). The inhibitory activity against HIV-$1_{HTLV-IIIB}$, therefore, can be taken as a general aspect of the anti-HIV potential of the individual compounds. The inhibitory activities of various groups of polyoxometalates against the syncytium formation catalyzed by HIV-$1_{SF-2H}$ or HIV-$1_{HTLV-IIIB}$ are comparatively shown in Table 4, in terms of the $IC_{50}$ values. There is some difference in the coverage between the following two

Table 1. Inhibition of HIV-$1_{HTLV-IIIB}$-Induced Cytopathogenicity by Polyoxometalates

| | Compound | $EC_{50}$ [a] (μg/ml) | $CC_{50}$ [b] (μg/ml) | SI [c] |
|---|---|---|---|---|
| Keggin structure | | | | |
| PM-92 | $H_4[SiW_{12}O_{40}]\cdot 24H_2O$ | 5.9 | 134 | 22.5 |
| PM-1 | $K_5[BW_{12}O_{40}]\cdot 15H_2O$ | 17.5 | 194 | 11.1 |
| PM-43 | $K_5[SiVW_{11}O_{40}]\cdot nH_2O$ | 4.8 | 180 | 37.4 |
| PM-44 | $K_5[PVW_{11}O_{40}]\cdot nH_2O$ | 21.5 | 135 | 6.3 |
| PM-47 | $K_7[BVW_{11}O_{40}]\cdot nH_2O$ | 9.9 | 102 | 10.3 |
| PM-83 | $K_5[SiFeW_{11}O_{39}(H_2O)]\cdot 14H_2O$ | 6.0 | 115 | 19.0 |
| PM-519 | $(iso\text{-}Pr_2NH_2)_5[PTiW_{11}O_{39}(O_2)]\cdot 4H_2O$ | 20.8 | 341 | 16.4 |
| PM-520 | $(iso\text{-}Pr_2NH_2)_5[PTiW_{11}O_{40}]\cdot 4H_2O$ | 17.0 | 389 | 22.8 |
| PM-19 | $K_7[PTi_2W_{10}O_{40}]\cdot 6H_2O$ | 3.9 | 138 | 34.9 |
| PM-77 | $(Me_4N)_7[PTi_2W_{10}O_{40}]\cdot nH_2O$ | 4.4 | 262 | 59.1 |
| PM-523 | $(iso\text{-}PrNH_3)_6H[PTi_2W_{10}O_{38}(O_2)_2]\cdot H_2O$ | 4.6 | 148 | 32.3 |
| PM-524 | $Cs_7[PTi_2W_{10}O_{38}(O_2)_2]\cdot nH_2O$ | 4.8 | 129 | 26.8 |
| PM-82 | $K_5[PV_2W_{10}O_{40}]\cdot nH_2O$ | 6.4 | 221 | 34.3 |
| PM-66 | $K_3[PMo_3W_9O_{40}]\cdot 25H_2O$ | — | 298 | — |
| PM-67 | $K_3[PMo_9W_3O_{40}]\cdot 5H_2O$ | — | 243 | — |
| PM-63 | $(NH_4)_6H[PZnMo_{11}O_{40}]\cdot 25H_2O$ | — | 180 | — |
| Lacunary Keggin structure | | | | |
| PM-65 | $K_7[PW_{11}O_{39}]\cdot nH_2O$ | 16.7 | 144 | 8.6 |
| PM-62 | $K_7[PMo_2W_9O_{39}]\cdot 19H_2O$ | — | 203 | — |
| Trivacant Keggin structure | | | | |
| PM-30 | $Na_9[SiW_9O_{33}(OH)]\cdot 23H_2O$ | 21.5 | 134 | 6.2 |
| PM-73 | $Na_{10}[SiW_9O_{34}]\cdot 18H_2O$ | 53.3 | 179 | 3.4 |
| PM-64 | $Na_3H_6[PMo_9O_{34}]\cdot 13H_2O$ | — | 344 | — |
| Keggin sandwich structure | | | | |
| PM-48 | $K_{13}[Eu(SiW_{11}O_{39})_2]\cdot nH_2O$ | 6.1 | 158 | 25.8 |
| Wells-Dawson structure | | | | |
| PM-29 | $K_8[P_2CoW_{17}O_{62}]\cdot 18H_2O$ | 7.1 | 51.4 | 7.2 |
| PM-510 | $K_8[P_2Mn^{2+}W_{17}O_{61}(H_2O)]\cdot nH_2O$ | 9.0 | 49.4 | 5.5 |
| Wells-Dawson sandwich structure | | | | |
| PM-526 | $K_{17}[Eu(P_2W_{17}O_{61})_2]\cdot nH_2O$ | 11.6 | 42.0 | 3.6 |
| Miscellaneous | | | | |
| PM-3 | $K_{27}[KAs_4W_{40}O_{140}]\cdot nH_2O$ | 13.5 | 73.1 | 5.4 |
| HPA-23 | $(NH_4)_{17}Na[NaSb_9W_{21}O_{86}]\cdot 14H_2O$ | — | 7.5 | — |
| PM-72 | $(NH_4)_{18}[(NH_4)Sb_9W_{21}O_{86}]\cdot nH_2O$ | — | 5.6 | — |
| PM-2 | $K_{18}[KSb_9W_{21}O_{86}]\cdot nH_2O$ | — | 27.7 | — |
| PM-525 | $Na_{10}[W_{12}O_{42}]\cdot nH_2O$ | — | 208 | — |
| PM-506 | $K_7[CoW_{11}O_{38}]\cdot 17H_2O$ | — | 131 | — |
| PM-511 | $(NH_4)_8[H_2Co_2W_{11}O_{40}]\cdot 20H_2O$ | — | 63.8 | — |
| PM-509 | $K_{13}[Cu_3(NO_3)(PW_9O_{34})_2]\cdot nH_2O$ | — | 86.6 | — |
| PM-527 | $K_{5.5}H_{1.5}[SbW_6O_{24}]\cdot 6H_2O$ | — | 209 | — |
| PM-113 | $(NH_4)_{12}H_2[Nd_4(MoO_4)(H_2O)_{16}(Mo_7O_{24})_4]\cdot nH_2O$ | — | 430 | — |
| PM-33 | $(NH_4)_6[Cr_2Mo_{12}O_{42}]\cdot 20H_2O$ | — | 368 | — |
| PM-5 | $Na_6[NiMo_9O_{32}]\cdot nH_2O$ | — | 14 | — |
| PM-8 | $(iso\text{-}PrNH_3)_6[Mo_7O_{24}]\cdot 3H_2O$ | — | 260 | — |
| PM-501 | $K_5H_2[V_{15}O_{36}(CO_3)]\cdot 6.5H_2O$ | — | 2.2 | — |
| PM-10 | $K_7[NiV_{13}O_{38}]\cdot 16H_2O$ | — | 0.5 | — |
| PM-932 | $(tert\text{-}BuNH_3)_4[V_4O_{12}]\cdot nH_2O$ | — | 2.4 | — |

Data represent mean values for two experiments. Cell viability was measured by the MTT metabolic assay 5 d after virus infection. *a)* 50% inhibitory concentration calculated on the basis of the reduction in the viability of mock-infected MT-4 cells. *b)* 50% effective concentration calculated on the basis of the inhibition of HIV-1-induced CPE in MT-4 cells. *c)* Selective index corresponding to the ratio $CC_{50}/EC_{50}$. *d)* Not available because the inhibition of CPE was less than 50% at non-toxic concentrations.

Table 2. Inhibition of HIV-$1_{SF-2H}$-Induced Cytopathogenicity by Polyoxometalates

| | Compound | $EC_{50}$ (μg/ml) | $CC_{50}$ (μg/ml) | SI |
|---|---|---|---|---|
| Keggin structure | | | | |
| PM-92 | $H_4[SiW_{12}O_{40}]\cdot nH_2O$ | 0.111 | 291 | 2620 |
| PM-43 | $K_5[SiVW_{11}O_{40}]\cdot nH_2O$ | 0.241 | 299 | 1240 |
| PM-44 | $K_5[PVW_{11}O_{40}]\cdot nH_2O$ | 2.93 | 310 | 106 |
| PM-519 | $(iso\text{-}Pr_2NH_2)_5[PTiW_{11}O_{39}(O_2)]\cdot 4H_2O$ | 0.343 | 469 | 1370 |
| PM-520 | $(iso\text{-}Pr_2NH_2)_5[PTiW_{11}O_{40}]\cdot 4H_2O$ | 0.319 | 476 | 1490 |
| PM-19 | $K_7[PTi_2W_{10}O_{40}]\cdot 6H_2O$ | 0.081 | 342 | 4230 |
| PM-77 | $(Me_4N)_7[PTi_2W_{10}O_{40}]\cdot nH_2O$ | 0.066 | 328 | 4970 |
| PM-523 | $(iso\text{-}PrNH_3)_6H[PTi_2W_{10}O_{38}(O_2)_2]\cdot H_2O$ | 0.047 | 310 | 6650 |
| PM-82 | $K_5[PV_2W_{10}O_{40}]\cdot nH_2O$ | 0.067 | 83.4 | 1250 |
| PM-66 | $K_3[PMo_3W_9O_{40}]\cdot 25H_2O$ | — | 362 | — |
| PM-67 | $K_3[PMo_9W_3O_{40}]\cdot 5H_2O$ | — | 331 | — |
| Lacunary Keggin | | | | |
| PM-65 | $K_7[PW_{11}O_{39}]\cdot nH_2O$ | 0.315 | 311 | 990 |
| PM-62 | $K_7[PMo_2W_9O_{39}]\cdot 19H_2O$ | — | 305 | — |
| Trivacant Keggin structure | | | | |
| PM-30 | $Na_9[SiW_9O_{33}(OH)]\cdot 23H_2O$ | 0.076 | 154 | 2020 |
| PM-73 | $Na_{10}[SiW_9O_{34}]\cdot 18H_2O$ | 0.221 | 339 | 1530 |
| Keggin sandwich structure | | | | |
| PM-48 | $K_{13}[Eu(SiW_{11}O_{39})_2]\cdot nH_2O$ | 0.234 | 346 | 1480 |
| Wells-Dawson structure | | | | |
| PM-29 | $K_8[P_2CoW_{17}O_{62}]\cdot 18H_2O$ | 1.31 | 64.0 | 48.7 |
| PM-510 | $K_8[P_2Mn^{2+}W_{17}O_{61}(H_2O)]\cdot nH_2O$ | 1.04 | 95.0 | 91.0 |
| Wells-Dawson sandwich structure | | | | |
| PM-526 | $K_{17}[Eu(P_2W_{17}O_{61})_2]\cdot nH_2O$ | 5.00 | 83.5 | 16.7 |
| Miscellaneous | | | | |
| PM-3 | $K_{27}[KAs_4W_{40}O_{140}]\cdot nH_2O$ | 125 | 333 | 2.67 |
| HPA-23 | $(NH_4)_{17}Na[NaSb_9W_{21}O_{86}]\cdot 14H_2O$ | — | 14.6 | — |
| PM-8 | $(iso\text{-}PrNH_3)_6[Mo_7O_{24}]\cdot 3H_2O$ | — | 370 | — |

Table 3. Strain Specificity in Inhibition of HIV-Dependent Syncytium Formation by PM-19

| HIV strain | $IC_{50}$ in μg/ml (Fusion index) |
|---|---|
| HIV-$1_{SF-2H}$ | 0.086 (0.68) |
| HIV-$1_{HTLV-IIIB}$ | 13.0 (0.72) |
| HIV-$1_{MN}$ | 9.57 (1.03) |
| HIV-$1_{RF}$ | 12.5 (1.81) |
| HIV-$2_{GH-1}$ | 2.48 (1.21) |
| HIV-$1_{SF-162}$ [a] | 4.91 (0.86) |

HIV-infected and uninfected MOLT-4 cells were cultured individually or in combination at the ratio of 1:2 for 20 h in the presence of PM-19. Each culture was established with $3.0 \times 10^5$ cells in a total volume of 1.2 ml. *a)* Vac-*env*-infected MOLT-4 cells and MT-2 cells were used instead of HIV-infected and uninfected MOLT-4 cells, respectively.

groups: (1) the compounds with a general anti-HIV-1 activity defined by the $EC_{50}$ for HIV-$1_{HTLV-IIIB}$ [$EC_{50}$ (HIV-$1_{HTLV-IIIB}$)] of less than 50 μg/ml (Table 1), and (2) the compounds highly specific for HIV-$1_{SF-2H}$ over HIV-$1_{HTLV-IIIB}$, with the ratios of $IC_{50}$ (HIV-$1_{HTLV-IIIB}$) to $IC_{50}$ (HIV-$1_{SF-2H}$) being higher than 50 (Table 2). The polyoxotungstates of such structural families as Wells-Dawson and Wells-Dawson sandwich are as potent as PM-19 with regard to anti-HIV-$1_{HTLV-IIIB}$ activity; however, the specificity of these compounds for HIV-$1_{SF-2H}$ is far less than that of PM-19.

The binding of polyclonal anti-HIV-1 antibodies to cells chronically infected by HIV-$1_{HTLV-IIIB}$ and HIV-$1_{SF-2H}$ (MOLT-4/HIV-$1_{HTLV-IIIB}$ and MOLT-4/HIV-$1_{SF-2H}$, respectively) were studied by flow cytometric analysis (Fig. 1). In the presence of PM-19 at 0.5 μg/ml, the binding of anti-HIV-1 antibodies was adversely affected in the case of MOLT-4/HIV-$1_{SF-2H}$ cells (Fig. 1a), but not in the case of MOLT-4/HIV-$1_{HTLV-IIIB}$ cells (Fig. 1b). In contrast, 50-μg/ml PM-19 inhibited antibody binding to both MOLT-4/HIV-$1_{HTLV-IIIB}$ and MOLT-4/HIV-$1_{SF-2H}$ cells (Fig. 1c, d), reflecting a general anti-HIV-1 activity.

## DISCUSSION

The results in this study are summarized in Table 5. Although some other HIV-1 strains such as HIV-$1_{RF}$ and HIV-$1_{MN}$ responded to polyoxometalates in a similar fashion to HIV-$1_{HTLV-IIIB}$, it remains to be addressed whether the marked susceptibility to polyoxometalates is a unique property of HIV-$1_{SF-2H}$, given the results for

Table 4. Inhibition of HIV-1-Dependent Cell Fusion by Polyoxometalates

| Compound | | $IC_{50}$[a] (μg/ml) | |
|---|---|---|---|
| | | HIV-$1_{SF-2H}$ | HIV-$1_{HTLV-IIIB}$ |
| Keggin structure | | | |
| PM-92 | $H_4[SiW_{12}O_{40}]\cdot 24H_2O$ | 0.074 | 24.7 |
| PM-1 | $K_5[BW_{12}O_{40}]\cdot 15H_2O$ | 0.254 | NT |
| PM-43 | $K_5[SiVW_{11}O_{40}]\cdot nH_2O$ | 0.033 | 9.43 |
| PM-44 | $K_5[PVW_{11}O_{40}]\cdot nH_2O$ | 0.107 | 30.4 |
| PM-47 | $K_7[BVW_{11}O_{40}]\cdot nH_2O$ | 0.067 | NT |
| PM-83 | $K_5[SiFeW_{11}O_{39}(H_2O)]\cdot 14H_2O$ | 0.093 | NT |
| PM-19 | $K_7[PTi_2W_{10}O_{40}]\cdot 6H_2O$ | 0.086 | 13.0 |
| PM-77 | $(MeN)_7[PTi_2W_{10}O_{40}]\cdot nH_2O$ | 0.018 | 6.82 |
| PM-82 | $K_5[PV_2W_{10}O_{40}]\cdot nH_2O$ | 0.081 | NT |
| PM-66 | $K_3[PW_9Mo_3O_{40}]\cdot 25H_2O$ | 35.6 | >250 |
| PM-67 | $K_3[PMo_9W_3O_{40}]\cdot 5H_2O$ | 60.8 | >250 |
| PM-63 | $(NH_4)_6H[PZnMo_{11}O_{40}]\cdot 25H_2O$ | 76.8 | NT |
| Lacunary Keggin structure | | | |
| PM-65 | $K_7[PW_{11}O_{39}]\cdot nH_2O$ | 0.021 | 39.3 |
| PM-62 | $K_7[PW_9Mo_2O_{39}]\cdot 19H_2O$ | 102 | NT |
| Trivacant Keggin structure | | | |
| PM-30 | $Na_9[SiW_9O_{33}(OH)]\cdot 23H_2O$ | 0.037 | 40.7 |
| PM-73 | $Na_{10}[SiW_9O_{34}]\cdot 18H_2O$ | 0.051 | 39.2 |
| PM-64 | $Na_3H_6[PMo_9O_{34}]\cdot 3H_2O$ | 24.3 | NT |
| Keggin sandwich structure | | | |
| PM-48 | $K_{13}[Eu(SiW_{11}O_{39})_2]\cdot 30H_2O$ | 0.159 | 12.1 |
| Wells-Dawson structure | | | |
| PM-29 | $K_{10}[P_2CoW_{17}O_{61}(H_2O)]\cdot 18H_2O$ | 0.976 | 15.5 |
| Wells-Dawson sandwich structure | | | |
| PM-526 | $K_{17}[Eu(P_2W_{17}O_{61})_2]\cdot nH_2O$ | 1.88 | 7.70 |
| Miscellaneous | | | |
| PM-3 | $K_{27}[KAs_4W_{40}O_{140}]\cdot nH_2O$ | 12.4 | 10.3 |
| HPA-23 | $(NH_4)_{17}Na[NaSb_9W_{21}O_{86}]\cdot 14H_2O$ | >250 | >250 |
| PM-72 | $(NH_4)_{18}[(NH_4)Sb_9W_{21}O_{86}]\cdot nH_2O$ | >250 | >250 |
| PM-2 | $K_{18}[KSb_9W_{21}O_{86}]\cdot nH_2O$ | >250 | NT |
| PM-113 | $(NH_4)_{12}H_2[Nd_4(MoO_4)(H_2O)_{16}(Mo_7O_{24})_4]\cdot nH_2O$ | >250 | NT |
| PM-5 | $Na_6[NiMo_9O_{32}]\cdot nH_2O$ | >250 | NT |
| PM-8 | $(iso\text{-}PrNH_3)_6[Mo_7O_{24}]\cdot 3H_2O$ | >250 | NT |
| PM-10 | $K_7[NiV_{13}O_{38}]\cdot 16H_2O$ | >250 | NT |

a) Concentration at which the fusion index was reduced by 50%.

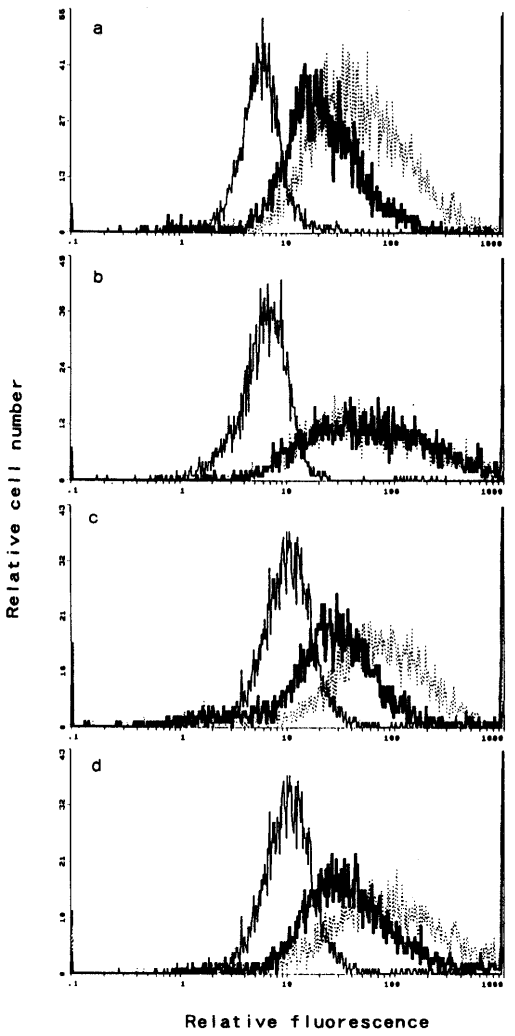


Fig. 1. Inhibition by PM-19 of the Binding of Polyclonal Anti-HIV-1 Antibodies to MOLT-4/HIV-$1_{SF-2H}$ and MOLT-4/HIV-$1_{HTLV-IIIB}$ Cells

The solid line histograms represent the background fluorescence of MOLT-4 cells. The broken line histograms show the fluorescence of untreated MOLT-4 cells chronically infected with HIV-$1_{SF-2H}$ (a, c) or HIV-$1_{HTLV-IIIB}$ (b, d), and the bold line histograms correspond to the HIV-1-infected MOLT-4 cells treated with 0.5 (a, b) or 50 μg/ml (c, d) PM-19.

a limited number of strains. Polyoxomolybdates and molybdic and tungstic polyoxometalates are devoid of anti-HIV-1 activity, even though they are in the same structural families as the active polyoxotungstates. In general, the compounds consisting of molybdates are chemically unstable because molybdates are easily reduced by mitochondria due to their lower redox potential compared with that of tungstates.

The mode of anti-HIV action of polyoxometalates is considered to be a blocked virion adsorption to membrane receptors or fusion-dependent penetration into target cells *via* interaction with the envelope glycoprotein gp120. The enhanced susceptibility of HIV-$1_{SF-2H}$ to polyoxometalates might be attributed to the tight interaction between polyoxometalates and HIV-$1_{SF-2H}$ gp120, as is evident from Fig. 1.

The anti-HIV-$1_{HTLV-IIIB}$ activity was shared by a rather wide range of polyoxotungstates, whereas the extent of specificity for HIV-$1_{SF-2H}$ over HIV-$1_{HTLV-IIIB}$ differed, even among these compounds. Especially, the compounds of structural families closely related to the Keggin structure were prone to be HIV-$1_{SF-2H}$ specific, while the strain specificity was not significant in the compounds of Wells-Dawson and Wells-Dawson sandwich families. The portion of Keggin structure recognized by gp120 of HIV-$1_{SF-2H}$ will be larger than the portion recognized by the other HIV-1 strains, including HIV-$1_{HTLV-IIIB}$. The former type of recognition will, in turn, result in a tight interaction with polyoxometalates, leading to a decrease in $EC_{50}$.

Table 5. Structure Requirement for Biological Activities of Polyoxometalates

| Structure | Anti-HIV activity | | Specificity for HIV-$1_{SF-2H}$[c] |
|---|---|---|---|
| | POT[a] | POM[b] | |
| Keggin | Positive | Negative | High |
| Lacunary Keggin | Positive | Negative | High |
| Trivacant Keggin | Positive | Negative | High |
| Keggin sandwich | Positive | | Moderate |
| Wells-Dawson | Positive | | Low |
| Wells-Dawson sandwich | Positive | | Low |
| Miscellaneous (except for PM-3) | Negative | Negative | — |

a) Polyoxotungstates. b) Polyoxomolybdates. c) The ratio of $IC_{50}$s for HIV-$1_{HTLV-IIIB}$ and HIV-$1_{SF-2H}$ in Table 3: high, >100; moderate, 50—100; low, ≦50.

A parental strain of HIV-$1_{SF-2H}$ (HIV-$1_{SF-2}$) and HIV-$1_{MN}$ are the most representative HIV strains found

in North America and Europe. However, HIV is notorious for making rapid changes in envelope glycoproteins, by which the susceptibility to polyoxometalates is determined, constantly creating new varieties of strains. Therefore, it will be of compelling interest to see wether polyoxometalates are active against isolates that have been freshly taken from patients.

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