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Intracellular metabolism and mechanism of anti-retrovirus action of 9-(2-phosphonylmethoxyethyl)adenine, a potent anti-human immunodeficiency virus compound

(reverse transcriptase/5-phosphoribosyl-1-pyrophosphate synthetase)

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ABSTRACT 9-(2-Phosphonylmethoxyethyl)adenine (PMEA) is a potent and selective inhibitor of retrovirus (i.e., human immunodeficiency virus) replication *in vitro* and *in vivo*. Uptake of PMEA by human MT-4 cells and subsequent conversion to the mono- and diphosphorylated metabolites (PMEAp and PMEApp) are dose-dependent and occur proportionally with the initial extracellular PMEA concentrations. Adenylate kinase is unable to phosphorylate PMEA. However, 5-phosphoribosyl-1-pyrophosphate synthetase directly converts PMEA to PMEApp with a K_m of 1.47 mM and a V_{max} that is 150-fold lower than the V_{max} for AMP. ATPase, 5′-phosphodiesterase, and nucleoside diphosphate kinase are able to dephosphorylate PMEApp to PMEAp, albeit to a much lower extent than the dephosphorylation of ATP. PMEApp has a relatively long intracellular half-life (16–18 hr) and has a much higher affinity for the human immunodeficiency virus-specified reverse transcriptase than for the cellular DNA polymerase α (K_i/K_m: 0.01 and 0.60, respectively). PMEApp is at least as potent an inhibitor of human immunodeficiency virus reverse transcriptase as 2′,3′-dideoxyadenosine 5′-triphosphate. Being an alternative substrate to dATP, PMEApp acts as a potent DNA chain terminator, and this may explain its anti-retrovirus activity.

Recently, we discovered a group of acyclic nucleoside phosphonate derivatives that exhibit a potent and selective inhibitory effect on the replication of human immunodeficiency virus (HIV) *in vitro* (1, 2). The prototype compound, 9-(2-phosphonylmethoxyethyl)adenine (PMEA), inhibits viral cytopathicity in MT-4 cells and viral antigen expression in HIV-1-, HIV-2-, and simian immunodeficiency virus-infected MT-4 and H9 cells at concentrations (i.e., 0.4–2.0 μM) that are well below the toxicity threshold for the host cells (i.e., 40–67 μM) (1–4). It has also proven effective in several experimental retrovirus infections in mice, cats, and monkeys (2–8). PMEA also protects mice against an acute lethal herpes simplex virus (HSV) infection (5, 9), has proved beneficial in the treatment of seropositive feline immunodeficiency virus-infected field cats with signs of opportunistic herpetic infections (8), and is effective against experimental equine herpes virus and cytomegalovirus (CMV) infection in mice (10, 11). PMEA is rather unique in that it has antiretroviral and antiherpetic properties and thus may be useful in AIDS patients for the treatment of the opportunistic herpesvirus (HSV, CMV, etc.) infections and the underlying retroviral disease.

Investigations have now been undertaken to gain a better insight in the cellular metabolism and mechanism of anti-HIV action of PMEA. PMEA has not been previously studied for its metabolic fate within the cell. Nor has its interaction with viral or cellular enzymes been examined. Our data have revealed that (*i*) PMEA may be directly converted intracellularly to its diphosphorylated derivative PMEApp, (*ii*) this phosphorylation is accomplished by 5-phosphoribosyl-1-pyrophosphate (PRPP) synthetase, (*iii*) PMEApp is targeted at the HIV reverse transcriptase (RT), and (*iv*) PMEApp acts as a chain terminator of the RT reaction.

MATERIALS AND METHODS

Cells. The origin and cultivation of MT-4 cells have been described (1, 2). MT-4 cells were a gift from N. Yamamoto (Yamaguchi University, Yamaguchi, Japan) and represent an immortalized helper/inducer T-cell clone obtained by cloning a normal T4 cell line in the presence of tumor cells derived from a patient with adult T-cell leukemia (12).

Compounds. PMEA was synthesized following a published procedure (13). [2,8-^{3}H]PMEA (specific radioactivity, 16 Ci/mmol; 1 Ci = 37 GBq) and [2,8-^{3}H]dATP (specific radioactivity, 24 Ci/mmol) were obtained from Moravek Biochemicals, Brea, CA. The diphosphate derivative of PMEA was prepared according to a published procedure (14).

Metabolism of [^{3}H]PMEA in Human MT-4 Cells. The metabolism of radiolabeled PMEA was monitored as follows: MT-4 cells were seeded at 2–4 × 10^5 cells per ml and incubated with different concentrations of [^{3}H]PMEA (varying from 0.5 to 312.5 μM). The total amount of radiolabel per cell culture was kept constant (10 μCi/ml). At different time intervals (i.e., 0, 24, 48, and 72 hr), cells were centrifuged at 4°C, thoroughly washed three times with ice-cold medium (without serum), and precipitated with 60% ice-cold methanol. After centrifugation at 10,000 rpm, the supernatants were filtered and quantitation of [^{3}H]PMEA and its metabolites was accomplished by HPLC analysis using a Partisil-SAX-10 radial compression column.

Intracellular Retention of [^{3}H]PMEA and Its Metabolites in MT-4 Cells After Removal of the Drug from the Culture Medium. MT-4 cells were seeded at 2–4 × 10^5 cells per ml and incubated with 0.5 μM [^{3}H]PMEA (50 μCi per 5-ml culture) for 24 hr. Then, the extracellular drug was removed by centrifugation of the cells and washing the cell cultures three times with warm culture medium. At 0, 24, and 48 hr after removal of [^{3}H]PMEA, cell extracts were prepared and [^{3}H]PMEA and its metabolites were determined by HPLC.

RT DNA Chain Termination Reaction by PMEApp and 2′,3′-Dideoxynucleotide 5′-Triphosphates (ddNTPs). M13 mp19 (+)-strand DNA, annealed to a 17-base M13 universal

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Abbreviations: HIV, human immunodeficiency virus; PMEA, 9-(2-phosphonylmethoxyethyl)adenine; PRPP, 5-phosphoribosyl-1-pyrophosphate; RT, reverse transcriptase; TCA, trichloroacetic acid; AZT, azidothymidine; AMV, avian myeloblastosis virus.

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primer, served as the template in a Sanger sequencing reaction using the RT of HIV or avian myeloblastosis virus (AMV). The reaction mixtures contained 60 mM Tris·HCl (pH 8.3), 75 mM NaCl, 7.5 mM $MgCl_2$, and 0.5 mM dithiothreitol. Separate termination mixtures (6 μl) were prepared for each 2′,3′-dideoxynucleotide. The termination mixtures contained 2.5 μl of either termination mixture A (1.2 μM ddATP/12 μM dATP/50 μM dCTP, dGTP, and dTTP), termination mixture C (1.2 μM ddCTP/12 μM dCTP/2 μM dATP/50 μM dGTP and dTTP), termination mixture G (1.2 μM ddGTP/12 μM dGTP/2 μM dATP/50 μM dCTP and dTTP), termination mixture T (1.2 μM ddTTP/12 μM dTTP/2 μM dATP/50 μM dCTP and dGTP), or termination mixture PMEApp (0.6 μM PMEApp/2 μM dATP/50 μM dCTP, dGTP, and dTTP) and 3.5 μl of sequencing buffer containing 0.5 μM dATP[^{35}S], 0.5 μM M13mp19 (+)-strand DNA, and 20 units of AMV RT or 16 units of HIV RT. After 5 min of incubation at 37°C, the reaction was terminated by adding 4 μl of a solution containing 95% (vol/vol) deionized formamide, 10 mM Na_2EDTA, 0.1% bromophenol blue, and 0.1% xylene cyanol FF. The termination reaction mixtures were heated to 80°C for 5 min and quenched on ice immediately prior to loading. Samples (2–3 μl) of each termination reaction mixture were then applied onto a 0.4-mm standard sequencing polyacrylamide gel [5.7% (wt/vol) acrylamide, 0.3% bisacrylamide, 0.1 M Tris·HCl (pH 8.3), 0.09 M boric acid, 1 mM Na_2EDTA, and 7 M urea]. Electrophoresis was performed for 1.5 hr at 1600 V. The gel was then fixed, dried, and exposed to an x-ray film at room temperature.

Enzyme Assays. Inhibition of recombinant HIV RT (p66) (generously supplied by P. J. Barr (Chiron) and calf thymus DNA polymerase α (Pharmacia) by PMEApp was determined as follows. In the HIV-1 RT assays in which the K_i value of PMEApp was determined, exogenous poly(U)·oligo(dA)$_{12-18}$ served as template. The reaction mixture (50 μl) contained 50 mM Tris·HCl (pH 7.8), 5 mM dithiothreitol, 300 mM glutathione, 500 μM EDTA, 150 mM KCl, 5 mM $MnCl_2$, 1.25 μg of bovine serum albumin, 2 μCi of [^{3}H]dATP (specific radioactivity, 24 Ci/mmol), 0.01 unit of poly(U)·oligo(dA)$_{12-18}$, 0.03% Triton X-100, 10 μl of PMEApp solution (containing various concentrations of the compound), and 1 μl of the RT preparation. The reaction mixtures were incubated at 37°C for 15 min, at which time 100 μl of calf thymus DNA (150 μg/ml), 2 ml of $Na_4P_2O_7$ (0.1 M in 1 M HCl), and 2 ml of trichloroacetic acid (TCA) (10%, vol/vol) were added. The solutions were kept on ice for 30 min, after which the acid-insoluble material was washed and analyzed for radioactivity.

In the HIV RT assays in which the effect of late addition of PMEApp was monitored, the [^{3}H]dATP concentration was invariably 2.5 μM. PMEApp (0.5 μM) was added at 3 min or 7.5 min after initiation of the reaction. The RT reaction in the absence or presence of the test compound was followed for 27 min and an aliquot was taken every 3 min for precipitation with 10% TCA and analysis of the acid-insoluble material for radioactivity.

In the calf thymus DNA polymerase α assays, activated calf thymus DNA (Pharmacia) served as the template. The reaction mixture (40 μl) contained 20 mM Tris·HCl (pH 7.9), 200 μM dithiothreitol, 3 mM $MgCl_2$, 20 μg of bovine serum albumin, 2 μg of activated DNA, 100 μM dGTP, dCTP, and dTTP, an appropriate concentration of [^{3}H]dATP, 8 μl of PMEApp (various concentrations) solution, and 12 μl of the DNA polymerase α preparation (diluted 40-fold). The reaction mixtures were incubated at 37°C for 30 min at which time 1 ml of 5% TCA was added. After 10 min on ice, the TCA-insoluble material was washed with 5% TCA, dried with 95% ethanol, and analyzed for radioactivity.

PRPP Synthetase Assays. Purified PRPP synthetase from *Escherichia coli* was obtained from Sigma. The reaction mixture (200 μl) contained 10 mM potassium phosphate buffer (pH 8.0), 5 mM $MgCl_2$, 2.5 mM PRPP, an appropriate amount of AMP or PMEA, and 0.04 unit of PRPP synthetase (in the PMEA assays) or 0.0002 unit of PRPP synthetase (in the AMP assays). The reaction mixtures were then incubated at 37°C for 10 min (AMP) or 4 hr (PMEA). During these incubation times, the reaction proceeded linearly. The assays were terminated by adding methanol at a final concentration of 60% to inactivate and remove the enzyme from the supernatant. The formation of ATP or PMEApp was analyzed by HPLC.

Table 1. Intracellular levels of [^{3}H]PMEA metabolites as a function of the initial (extracellular) PMEA concentration after 24 hr of incubation

Initial (extracellular) PMEA, μM	Intracellular level, nmol per 10^9 cells		
	PMEA	PMEAp	PMEApp
0.5	0.02 ± 0.025	0.010 ± 0.003	0.007 ± 0.001
2.5	0.546 ± 0.173	0.046 ± 0.001	0.037 ± 0.003
12.5	3.16 ± 1.05	0.313 ± 0.045	0.162 ± 0.005
62.5	14.6 ± 0.53	0.956 ± 0.163	1.021 ± 0.166
312.5	102 ± 3.53	4.97 ± 0.713	5.44 ± 0.237

RESULTS

Phosphorylation of [^{3}H]PMEA in MT-4 Cells as a Function of Different Input Concentrations. Formation of the monophosphorylated (PMEAp) and diphosphorylated (PMEApp) derivatives of PMEA increased proportionally with higher input concentrations (Table 1). At an initial PMEA concentration of 0.5 μM, 92 pmol of [^{3}H]PMEA per 10^9 MT-4 cells

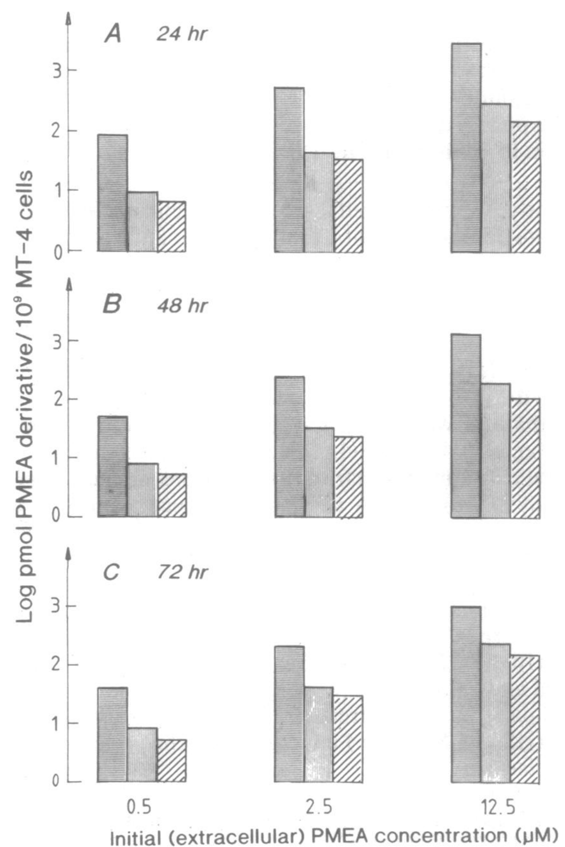

FIG. 1. Intracellular amounts of [^{3}H]PMEA (≡), [^{3}H]PMEAp (|||), and [^{3}H]PMEApp (⫽) following incubation of MT-4 cells for 24 hr (*A*), 48 hr (*B*), or 72 hr (*C*) with different (initial) concentrations of [^{3}H]PMEA.

appeared intracellularly within 24 hr of incubation, and 10-fold lower concentrations of PMEAp and PMEApp were recorded. A serial (5-fold) increase in the initial extracellular concentration of PMEA resulted in a concomitant increase in the intracellular amounts of PMEA, PMEAp, and PMEApp. As a rule, intracellular PMEAp and PMEApp levels were invariably 10- to 20-fold lower than intracellular PMEA levels, irrespective of the initial (extracellular) concentration of PMEA (Table 1). We have recently shown that [^{3}H]PMEA is not taken up by the cells by the temperature-insensitive nucleoside carrier-mediated transport but by an endocytosis-like process that occurs at 37°C but could be efficiently blocked at 4°C (15). Thus, by keeping all cell washings and centrifugation steps before the extraction of the cells at 4°C, we ascertained that no [^{3}H]PMEA wasa diffused back to the supernatant and avoided the risk of underestimating the intracellular [^{3}H]PMEA content.

Phosphorylation of [^{3}H]PMEA in MT-4 Cells as a Function of Different Incubation Times. Upon incubation of MT-4 cells with 0.5 μM PMEA, the intracellular PMEA levels measured after 24 hr reached 92 pmol per 10^9 cells (intracellular concentration, $\approx$0.09 μM) and decreased to 42 pmol per 10^9 cells after 72 hr (Fig. 1). The intracellular levels of PMEAp and PMEApp following a 24-hr incubation period were 9.9 and 7.3 pmol per 10^9 cells, respectively, and these levels were not markedly changed if the incubation time was extended to 48 or 72 hr. Similar findings were obtained at higher PMEA input concentrations (i.e., 2.5 and 12.5 μM). Thus, although the intracellular PMEA levels decreased to <50% if the incubation time was extended from 24 to 72 hr, the levels of the phosphorylated PMEA metabolites only slightly decreased upon prolonging the incubation period (Fig. 1).

Retention of the Intracellular [^{3}H]PMEA Metabolite Levels upon Removal of PMEA from the Culture Medium. MT-4 cells were incubated with 0.5 μM [^{3}H]PMEA for a 24-hr period, upon which the drug was removed from the extracellular medium. At 24 hr after removal of [^{3}H]PMEA, the intracellular levels of PMEA had decreased by 87%, and the PMEAp and PMEApp levels had decreased by 65% (data not shown). At 48 hr after removal of [^{3}H]PMEA, the intracellular levels of PMEA, PMEAp, and PMEApp had decreased by 90–97%. Based on these findings, the initial intracellular half-life for PMEApp could be estimated at 16–18 hr.

Inhibitory Effect of PMEApp on Recombinant HIV-1 p66 RT and Calf Thymus DNA Polymerase α. PMEApp proved strongly inhibitory to HIV-1 RT. With poly(U)·oligo(dA) as the exogenous template/primer and [^{3}H]dATP (3 μM) as the natural substrate, the 50% inhibitory concentration (IC_{50}) of PMEApp for HIV-1 RT was 0.18 μM. In contrast, PMEApp was much less inhibitory to cellular DNA polymerase α (IC_{50}, 1.8 μM). PMEA as such had no effect on both enzymes, even at a concentration of 500 μM. When evaluated against HIV-1 RT and DNA polymerase α at different [^{3}H]dATP concentrations, PMEApp showed a K_i of 0.09 μM for HIV-1 RT and 2.14 μM for DNA polymerase α. Its K_i/K_m ratio for HIV-1 RT and DNA polymerase α was 0.01 and 0.60, respectively. The inhibition of HIV-1 RT and DNA polymerase α by PMEApp, as evident from the Lineweaver–Burk diagram, was competitive with respect to [^{3}H]dATP (data not shown).

Effect of Preincubation of HIV-1 RT with PMEApp and Delayed Addition of PMEApp to the HIV-1 RT Reaction. Preincubation of HIV-1 RT with PMEApp (0.25 μM or 0.75 μM) for 1–5 min before addition of [^{3}H]dATP resulted in a significant decrease of the HIV-1 RT activity. Following

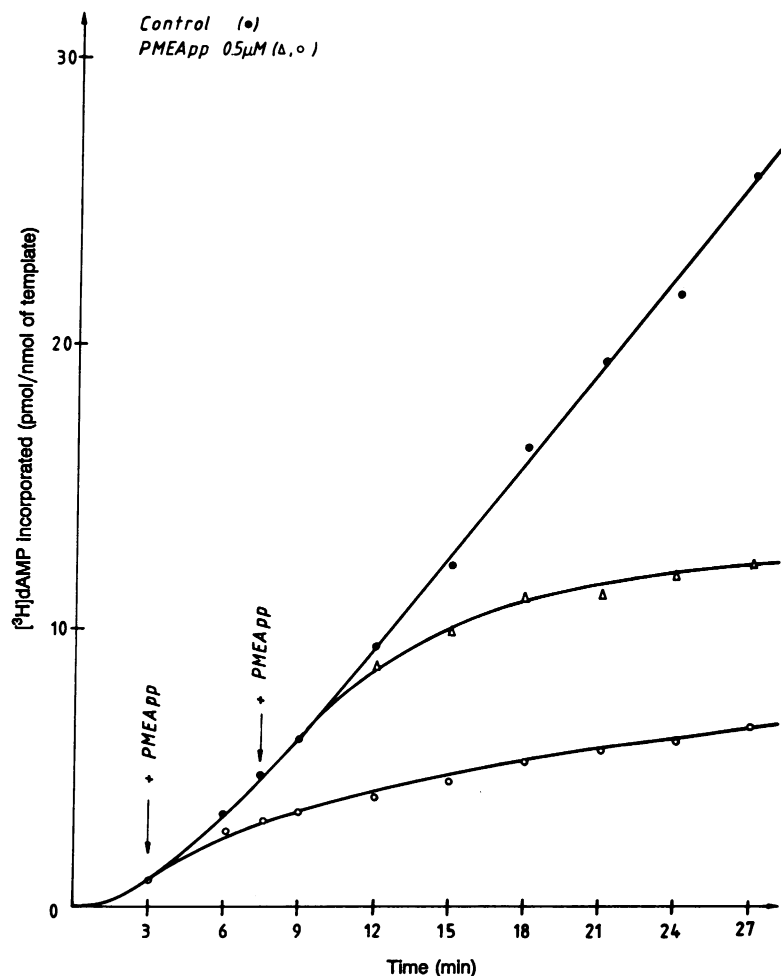

FIG. 2. Effect of 0.5 μM PMEApp on the poly(U)·oligo(dA)-directed incorporation of [^{3}H]dAMP by HIV-1 RT. PMEApp was added to the reaction mixture at 3 or 7.5 min after initiation of the reaction. The [^{3}H]dATP concentration was 3 μM.

preincubation with 0.75 or 0.25 μM PMEApp, poly(U)·oligo-(dA)-directed incorporation of [^{3}H]dAMP into DNA was decreased to 40% or 60%, respectively, of the incorporation rates obtained with PMEApp added at the initiation of the reaction (data not shown).

In a second set of experiments, 0.5 μM PMEApp was added at 3 and 7.5 min after initiation of the RT reaction (Fig. 2). Immediately after PMEApp had been added, the RT reaction ceased to proceed linearly and leveled off almost completely (Fig. 2). When poly(A)·oligo(dT) is used as the template/primer, [^{3}H]dTMP incorporation is not affected by the addition of PMEApp (data not shown).

DNA Chain Termination by PMEApp and ddATP. PMEApp and ddATP were compared for their DNA chain-terminating effects in a Sanger sequencing reaction using an M13 mp19 (+)-strand DNA:17-base M13 template/primer and the RTs from either HIV or AMV (Fig. 3). The 2′,3′-dideoxynucleotides ddATP, ddCTP, ddGTP, and ddTTP act as potent DNA chain-terminating agents in the RT reaction when present at 10-fold lower concentrations than their natural 2′-deoxynucleotide counterparts (Fig. 3). PMEApp proved almost equally inhibitory to the DNA polymerization reaction as ddATP. DNA chain termination by PMEApp occurred at the same sites where ddATP caused DNA chain termination (Fig. 3).

Interaction of PMEA with 5′-Nucleotidase, Adenosine Deaminase (ADA), Adenosine 5′-Monophosphate (AMP) Deaminase, AMP Kinase, and PRPP Synthetase. PMEA was examined as a potential substrate/inhibitor for 5′-nucleotidase, ADA, rabbit muscle AMP deaminase, AMP kinase (myokinase) (using ATP, dATP, and GTP as the phosphate donor), and PRPP synthetase. None of the enzymes was markedly inhibited in the presence of PMEA at a concentration of 500 μM, except for PRPP synthetase. Moreover, unlike AMP, which was readily converted to IMP by rabbit muscle AMP deaminase or ADP by porcine muscle AMP kinase, PMEA did not act as a substrate for either enzyme under experimental conditions where 100-fold more enzyme was used than required to completely convert AMP to IMP or ADP within a few minutes (data not shown). However, PMEA was converted to PMEApp by purified PRPP synthetase. The K_m values of PRPP synthetase for AMP and PMEA were 0.178 mM and 1.47 mM, respectively. The V_{max} values were 13,000 and 87 nmol/unit of enzyme per hr, respectively. Thus, PMEA was directly converted to PMEApp by PRPP synthetase at an 8-fold higher K_m and a 150-fold lower V_{max} than the natural substrate AMP.

DISCUSSION

PMEA is a potent and selective inhibitor of the replication of several retroviruses (i.e., HIV-1, HIV-2, simian immunodeficiency virus, feline immunodeficiency virus, murine sarcoma virus) *in vitro* and *in vivo*. Our data clearly point to the RT as the molecular target for the antiviral action of PMEA. The diphosphorylated product of PMEA, termed PMEApp, is a potent inhibitor of HIV-1 RT (K_i/K_m, 0.01), and, like ddATP, it also acts as a DNA chain terminator at the dAMP sites (Figs. 2 and 3). Although the kinetics of the PMEApp-inhibited RT reaction during the time course of 15 min are nonlinear (in contrast to the linearity obtained in the uninhibited RT reaction) (Fig. 2), no markedly different K_i/K_m values were obtained when the RT reaction was followed for only 5 min (K_i/K_m, 0.01–0.02). These observations indicate that inactivation of the template-primer by PMEApp is less pronounced than the competitive inhibitory effect of PMEApp (with respect to dATP) on the enzyme under our experimental conditions. This may be essentially due to the excess of template-primer in the RT reaction mixture. Our experiments also revealed that PMEApp does not inactivate the enzyme *per se*. It does not interfere with the initiation of the RT reaction but rather affects DNA elongation. [^{3}H]-PMEA is incorporated as such at the 3′ end of the DNA. This was verified by hydrolysis of the DNA by $HClO_4$ and analysis of the products by HPLC.

The metabolism of PMEA clearly differs from that of 2′,3′-dideoxyadenosine (16–18) in that it shows a straightforward pattern of intracellular phosphorylation. No marked intracellular release of free adenine and no significant deamination to the hypoxanthine derivative [^{3}H]PMEHx was found, even when MT-4 cells were incubated with [^{3}H]PMEA for a prolonged period. However, it cannot be excluded that PMEA is deaminated in cell systems other than MT-4. Indeed, a limited amount of the hypoxanthine derivative of PMEA has been observed in PMEA-treated HeLa cell cultures (Y.-C. Cheng, personal communication).

Relatively low amounts of PMEAp and PMEApp are formed from PMEA inside the MT-4 cells. At equimolar extracellular concentrations (i.e., 10 μM initially), azidothymidine (AZT) and D4T reach intracellular AZT and D4T 5′-triphosphate concentrations of 1.1 and 1.0 nmol per 10^9 cells, respectively (19), compared with ±0.14 nmol per 10^9

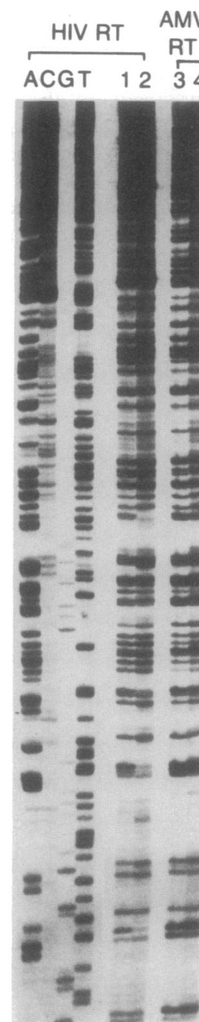

FIG. 3. Sanger sequencing reaction with HIV-1 or AMV RT. Lanes A, C, G, and T, DNA chain termination by ddATP, ddCTP, ddGTP, and ddTTP, respectively. Lanes 1 and 3, DNA chain termination by ddATP for HIV-1 RT and AMV RT, respectively. Lanes 2 and 4, DNA chain termination by PMEApp for HIV-1 RT and AMV RT, respectively. Note that the bands in lanes A, C, G, and T are located slightly lower than the corresponding bands in lanes 1 and 2 and lanes 3 and 4.

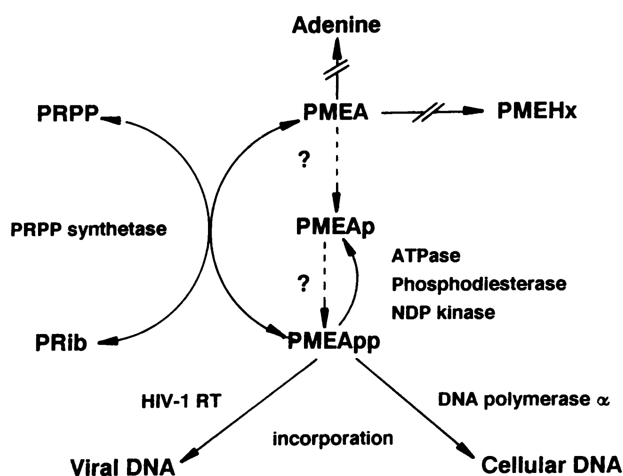

FIG. 4. Intracellular metabolism of PMEA in MT-4 cells.

cells for PMEApp (Table 1). However, when the D4T and AZT 5′-triphosphate levels are compared with the PMEApp levels at equivalent antivirally effective doses in MT-4 cells (i.e., 0.006, 0.2, and 5 μM for AZT, D4T, and PMEA, respectively), AZT 5′-triphosphate, D4T 5′-triphosphate, and PMEApp levels are 0.01, 0.06, and 0.07 nmol per 10^9 cells after a 24-hr incubation period (ref. 19 and Table 1). Thus, PMEApp levels obtained within 24 hr are comparable to D4T 5′-triphosphate levels at equivalent antivirally effective doses.

We found that PMEA could be directly converted to PMEApp through pyrophosphate transfer from PRPP. This reaction is catalyzed by PRPP synthetase, an enzyme that is known to have a very stringent substrate specificity and mainly functions in the "catabolic direction" whereby PRPP and AMP are formed starting from ATP and 5-phosphoribose. In addition to the natural substrates ATP and 5-phosphoribose, only 5-amino-4-imidazolecarboxamide riboside triphosphate and 7-deaza-AMP (tubercidin 5′-monophosphate) have ever been reported to act as alternative substrates for this enzyme (20). PRPP synthetase normally proceeds in the direction of AMP synthesis at an equilibrium ratio of ±9:1 (20). The fact that invariably higher intracellular concentrations are reached for PMEA than PMEApp may be consistent with these kinetic properties of the enzyme and/or with the relatively high K_m (1.47 mM) of PRPP synthetase for PMEA. The extremely low V_{max} of PRPP synthetase for the conversion of PMEA to PMEApp may not only account for the slow synthesis of PMEApp from PMEA but also for the relatively long intracellular retention time of PMEApp after removing PMEA from the extracellular medium. In this respect, it should be noted that PMEApp also has a much lower substrate affinity than ATP for ATP-catabolizing enzymes, including ATPase, 5′-phosphodiesterase, and nucleoside diphosphate kinase (data not shown), and this, in turn, may explain why low levels of PMEAp are generated within PMEA-treated cells (Table 1, Fig. 1). Since the kinetic data found for PMEA against PRPP synthetase are obtained from enzyme experiments in cell-free assays, the exact role of PRPP synthetase in the phosphorylation of PMEA in intact cells should still be clarified. It cannot be excluded that enzymes other than PRPP synthetase may play an important role in the conversion of PMEA to its antivirally active metabolite PMEApp.

PMEAp and PMEApp have a relatively long intracellular half-life. This property contrasts with the short half-life of the 5′-triphosphate derivatives of the pyrimidine 2′,3′-dideoxyribosides AZT and D4T (21) but resembles the half-life of the 5′-triphosphate derivative of the purine 2′,3′-dideoxynucleoside ddATP (16). The long intracellular half-life may be the reason for the pronounced anti-retrovirus effects of PMEA *in vitro* and *in vivo*.

In conclusion (Fig. 4), PMEA is postulated to follow a unique metabolic pathway within the MT-4 cells. Although not a substrate for purine nucleotide kinase(s) or adenosine (or AMP) deaminases, PMEA could be recognized as a substrate for PRPP synthetase that converts it directly to PMEApp, which, on the one hand, could be dephosphorylated by several enzymes to PMEAp, and, on the other hand, would represent the active form of PMEA. PMEApp could serve as a competitive inhibitor/alternative substrate of the RT reaction and, if incorporated, act as a DNA chain terminator.

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