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# Dual biological activity of apurinic acid on human lymphocytes: induction of interferon-γ and protection from human immunodeficiency virus infection in vitro

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## Summary

The chemically modified DNA, apurinic acid (APA), is cytotoxic for human lymphocytes at concentrations above 100 μg/ml. At low concentrations (0.05–1 μg/ml) APA acts as an inducer of interferon γ (IFN-γ) in lymphocytes in vitro; the maximum interferon titer of 50 units/ml was reached at 0.4 μg/ml. When added to the cells in combination with phytohemagglutinin A (PHA), APA displays a significant synergistic interferon-inducing ability; the maximum titer of 940 units/ml was obtained with 10 μg/ml of APA and 6.25 μg/ml of PHA. APA also proved to be an effective inhibitor of human immunodeficiency virus (HIV-1) replication in H9 cells. At a concentration of 10 μg/ml, APA causes a 49% inhibition of virus growth, while 20 μg/ml of APA are required to inhibit expression of HIV-1 p17 and p24 *gag* proteins by 60%. The mechanism of anti HIV-1 activity of APA likely occurs at the level of viral reverse transcriptase. This enzyme is inhibited by APA

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*Abbreviations*: AIDS, acquired immunodeficiency syndrome; APA, apurinic acid; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; HIV, human immunodeficiency virus; HTLV-III, human T-lymphotropic virus; IFN-γ, γ-interferon; IL-2, interleukin 2; $K_i$, enzyme inhibition constant; PHA, phytohemagglutinin A; PWM, pokeweed mitogen; RT, reverse transcriptase.

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in a noncompetitive way with a $K_i$ of 0.39 μM, while the cellular DNA polymerases α, β and γ are 140- to 300-fold less sensitive to APA.

Apurinic acid; Human immunodeficiency virus; Interferon γ; Reverse transcriptase

## Introduction

The etiologic agent of acquired immunodeficiency syndrome (AIDS) has been identified as a human immunodeficiency virus (HIV-1), also known as human T-cell lymphotropic virus III (HTLV-III) or lymphoadenopathy-associated virus (Gallo et al., 1984; Barré-Sinoussi et al., 1983). HIV belongs to the class of retroviruses, lacking transforming genes; they contain in addition to the viral replication genes (*gag, pol, env*) at least five extra genes (Wong-Staal and Gallo, 1985; Wong-Staal et al., 1987). The latter genes are involved in the regulation of HIV expression at the levels of transcription, processing, translation and virus maturation (Wong-Staal and Gallo, 1985). At present the following strategies for a pharmacological intervention against HIV have been applied: Inhibition of reverse transcriptase (RT) by competitive (e.g. 3′-azido-3′-deoxythymidine [Mitsuya et al., 1985]) or non-competitive compounds (e.g. phosphonoformic acid [Sarin et al., 1985; Wondrak et al., 1988]), as well as by synthetic oligonucleotides, complementary to viral RNA template (Zamecnik et al., 1986); inhibition of the expression of viral protease gene (W.E.G. Müller et al., to be published); and inhibition of viral budding by interferons (IFN) (Ho et al., 1985). The anti-HIV effect of IFN has been obtained both after application of exogenous IFN (Ho et al., 1985) and after induction of this immunoregulator by mismatched double-stranded RNA (Montefiori et al., 1987).

Here we describe that the chemically modified DNA, apurinic acid (APA), displays a novel, dual biological activity, (*i*) it inhibits HIV RT, and in consequence replication of HIV, and (*ii*) it induces IFN-γ.

## Materials and Methods

*Materials*

[*Methyl*-$^3$H]thymidine (dThd; spec. act. 87 Ci/mmol), [$^3$H]dGTP (spec. act. 20 Ci/mmol) and [$^3$H]dTTP (spec. act. 40 Ci/mmol) was obtained from the Radiochemical Centre, Amersham, U.K.; pokeweed mitogen (no. L 9379 [PWM]) from Sigma, St. Louis, MO, U.S.A.; phytohemagglutinin A (no. HA 17 [PHA]) from Deutsche Wellcome, Burgwedel, F.R.G.; oligo(dT)-cellulose from Collaborative Research, Waltham, MA, U.S.A.; the primer/templates: poly(dC)·$(dG)_{12\text{-}18}$, poly(dA)·$(dT)_{10}$, poly(A)·$(dT)_{10}$ and poly(C)·$(dG)_{18}$ from Pharmacia, Freiburg, F.R.G.

*Apurinic acid*

APA was prepared from herring sperm DNA (Tamm et al., 1952). The freshly prepared APA preparation contained: adenine, 0%; guanine, 0%; thymine, 28.6%; and cytosine and 5-methylcytosine, 21.4% (Heicke, 1970). The molecular weight of this nonreduced preparation was $1.9 \pm 0.5 \times 10^4$ daltons (Müller et al., 1973). Where indicated, a reduced APA preparation was used; the reduction of the aldehyde groups present in APA was achieved as described (Wyatt, 1951). Low molecular fragments of APA (fraction IV) were obtained by sonication followed by gel filtration (Müller et al., 1973).

*HTLV-IIIB and H9 cells*

HTLV-IIIB was obtained from culture supernatants of virus producing H9 cells (Popović et al., 1984). H9 cells were maintained in RPMI-1640 medium containing 20% fetal calf serum. $5 \times 10^5$ H9 cells were infected with $5 \times 10^8$ HTLV-IIIB particles and cultivated for 4 days in the absence or presence of APA (Sarin et al., 1987). Then the total viable cells per assay as well as the percentage of cells expressing p24 and p17 *gag* proteins of HIV-1 was determined by indirect immunofluorescence microscopy with the use of mouse monoclonal antibodies to HIV-1 p17 and p24. The positive cells were visualized by treatment with fluorescein-labeled goat anti-mouse IgG (Poiesz et al., 1980). The reactivity of the monoclonal antibodies with HIV-1-infected H9 cells was in the range of 20–30%. As a measure of viral titers the activity of reverse transcriptase (RT) was determined in the supernatants of the cultures (Poiesz et al., 1980) after precipitating the virus to remove APA (Popović et al., 1984). The assay of RT activity was performed as described (Sarin et al., 1987).

*Cultivation of human peripheral blood lymphocytes*

Peripheral blood lymphocytes were isolated from buffy coats of normal donors (blood bank, Bad Kreuznach, F.R.G) by sedimentation on Ficoll-Hypaque (Böyum, 1968). They were washed twice with RPMI-1640 medium. Then the cells were suspended at a density of $5 \times 10^6$ cells/ml in RPMI-1640 medium containing 10% fetal calf serum, supplemented with 1% of glutamine and antibiotics (penicillin and streptomycin). 1-ml cultures were cultivated at 37°C under humidified 5% $CO_2$/95% air for 0–72 h.

*Treatment of lymphocytes*

For the determination of the effect of APA on [$^3$H]-dThd incorporation into DNA the cultures were incubated for 72 h in the presence of different concentrations of APA. Eighteen hours prior to the end of the incubation 0.5 μCi of [$^3$H]-dThd was added to each cup. Where indicated, 6.25 μg/ml of PHA or 3 μg/ml of PWM were added to the cultures. APA was dissolved in RPMI-1640 medium and added at time zero to the cultures. Incorporation of [$^3$H]-dThd was determined as described (Leyhausen et al., 1984). Each value came from six parallel experiments. The $ED_{50}$ concentrations causing a 50% reduction of [$^3$H]-dThd incorporation were estimated by logit regression (Sachs, 1984).

The influence of APA and PHA on the production of IFN-γ was determined as follows: 1-ml cultures were incubated for 0–72 h in the presence of (*a*) different concentrations of APA, (*b*) 6.25 μg/ml PHA, and (*c*) APA plus PHA (6.25 μg/ml). The cultures were then centrifuged, the supernatants were collected and the IFN-γ titer was determined.

*Interferon assay*

IFN-γ was assayed by the enzyme-immuno-assay as described (Gallati, 1982). Briefly, 96-well flat-bottom plates (Greiner, Nürtingen; F.R.G.) were coated with anti-human IFN-γ. This monoclonal antibody did not cross-react with IFN-α or IFN-β. 50 μl of culture supernatants were then added per well together with 50 μl of peroxidase-labeled anti-human IFN-γ. After 24 h at 4°C the wells were washed with phosphate buffered saline. Finally, the peroxidase reaction was performed and the absorbance at 450 nm was determined (Bachmann et al., 1986). The calibration curve for the quantitation of the IFN-γ titer in the supernatants was obtained by using recombinant standard IFN-γ; the activity is expressed as IFN-γ NIH-units/ml of culture supernatants. The curve was linear between 0–200 NIH-units/ml.

*RNA isolation and blot hybridization*

Total RNA from HTLV-IIIB-infected H9 cells, grown in the absence or presence of APA, was extracted (Chirgwin et al., 1979). Subsequently, poly(A)-rich mRNA was isolated from total RNA by oligo(dT)-cellulose chromatography (Maniatis et al., 1982). The concentration of the extracted RNA was calculated (1 $O.D._{260}$ = 37.1 μg RNA/ml).

For Northern blot hybridization, poly(A)-rich mRNA was denatured at 56°C for 30 min in electrophoresis buffer (40 mM morpholinopropanesulfonic acid, 10 mM sodium acetate, 1 mM EDTA; pH 7.2) containing 50% dimethyl sulfoxide and 6% formaldehyde (Goldberg, 1980) and subjected to electrophoresis on 1.1% agarose gels containing 6% formaldehyde (Maniatis et al., 1982). The separated RNAs were blot transferred to nitrocellulose (BA 85; Schleicher and Schuell) and hybridized with the $^{32}$P-labeled probe (Maniatis et al., 1982) under the conditions described earlier (Messer et al., 1986). Exposition of the dry nitrocellulose filters to Kodak XAR-5 X-ray film (Eastman Kodak) backed by one intensifying screen was done at −70°C for 4 days.

The probe consisting of the HTLV-IIIB 3′-*orf*/LTR fragment, with the polyadenylation site (nucleotide position 8475 [*Xho*I restriction site] at position 9194 [*Hin*dIII site] according to nucleotide sequence of HTLV-IIIB published by Ratner et al. (1985)), was cloned into the pUC-vector; it was nick-translated to a specific activity of $7 \times 10^7$ cpm/μg DNA (Rigby et al., 1977).

*Polymerases and enzyme assays*

DNA polymerase α was isolated and partially purified from calf thymus as described by Bollum et al. (1974). The DNA polymerase α pool obtained from DE-11 cellulose column was further fractionated by ammonium sulfate precipitation and Sephadex G-200 gel filtration (Chang and Bollum, 1981). The specific activity

of the final preparation was 2340 units/mg. DNA polymerase β was purified from calf thymus by phosphocellulose column chromatography, gel filtration and affinity chromatography on DNA cellulose (Chang, 1973). The specific activity was 54 500 units/mg. DNA polymerase γ was isolated and purified from rat liver mitochondria by DEAE-cellulose- and phosphocellulose column chromatography (Bolden et al., 1977). The specific activity was determined to be 12.1 units/mg. HIV-1 reverse transcriptase was isolated from HTLV-IIIB-infected H9 cells by successive chromatography on DEAE-cellulose and phosphocellulose columns (Vogel and Chandra, 1981). The specific activity was 13.2 units/mg [using poly(A)·$(dT)_{10}$ as template-primer] and 54.9 units/ml [poly(C)·$(dG)_{18}$], resp. One unit of enzyme is defined as 1 nmol of total nucleotide polymerized/h.

DNA polymerase activities were determined essentially as described (Wondrak et al., 1988). The standard reaction system (final volume : 100 µl) contained the following components; DNA polymerase α: 50 mM Tris-HCl, pH 8.2, 1 mM DTT, 0.01% NP 40, 25 mM KCl, 5 mM $MgCl_2$, 0.2 OD/ml poly(dC)·$(dG)_{12\text{-}18}$, 16 µM [$^3$H]dGTP (400 dpm/pmol); DNA polymerase β: 50 mM Tris-HCl, pH 8.2, 1 mM DTT, 0.01% NP 40, 100 mM KCl, 0.5 mM $MnCl_2$, 0.2 OD/ml poly(dA)· $(dT)_{10}$, 16 µM [$^3$H]dTTP (200 dpm/pmol); DNA polymerase γ: 50 mM Tris-HCl, pH 8.2, 1 mM DTT, 0.01% NP 40, 100 mM KCl, 0.5 mM $MnCl_2$, 0.2 OD/ml poly(A)· $(dT)_{10}$, 16 µM [$^3$H]dTTP (200 dpm/pmol) and HIV-1 RT: 50 mM Tris-HCl, pH 8.2, 1 mM DTT, 0.01% NP 40, 100 mM KCl, 5 mM $MgCl_2$, 0.2 OD/ml poly(A)· $(dT)_{10}$ [or poly(C)·$(dG)_{18}$], 16 µM [$^3$H]dTTP (300 dpm/pmol [or [$^3$H]dGTP (300 dpm/pmol)]. Incubations were performed at 37°C for 1 h; acid-insoluble radioactivity was determined (Arya et al., 1985). During that period of time the reaction kinetics of the different enzymes used were linear. The enzyme inhibition studies were performed as described earlier (Müller et al., 1973) and the type of inhibition as well as the $K_i$ values were determined according to the method of Lineweaver and Burk (Lineweaver and Burk, 1934).

*Statistical evaluation*

Student's *t* test was applied to determine the significance of the IFN-γ production (Sachs, 1984).

## Results

*Induction of IFN-γ by APA*

*Effect of APA on [$^3$H]-dThd incorporation into lymphocytes.* In the absence of APA the [$^3$H]-dThd incorporation rates into the lymphocytes (after an 18-h incubation period with [$^3$H]-dThd) were determined to be: $1.3 \pm 0.1 \times 10^3$ dpm/$10^6$ cells (without mitogen), $38.2 \pm 2.3 \times 10^3$ dpm/$10^6$ cells (in the presence of 6.25 µg/ml of PHA) or $18.9 \pm 1.2 \times 10^3$ dpm/$10^6$ cells (in the presence of 3 µg/ml of PWM).

In the presence of 30 µg/ml of APA for an incubation period of 72 h no significant influence on the [$^3$H]-dThd incorporation rates was observed (Fig. 1). Higher

concentrations are required to cause inhibition. The following $ED_{50}$ concentrations were estimated for APA from the dose-response experiments (Fig. 1): in the absence of mitogen, 167 ± 12 μg/ml; in the presence of PHA, 112 ± 9 μg/ml and in the presence of PWM, 125 ± 9 μg/ml.

*IFN-γ production by lymphocytes after incubation with APA or PHA.* APA at a concentration as low as 0.07 μg/ml caused a significant production of IFN-γ in lymphocytes (*P* value versus control; <0.005) : 19 units/ml after a 24-h incubation period and 36 units/ml after 72 h. The production increases in 72 h cultures to 49 units/ml at an APA concentration of 0.4 μg/ml. Higher APA concentrations were less stimulatory. In the absence of APA or PHA lymphocytes produced only little IFN-γ: 1 ± 1 unit/ml after 24 h and 6 ± 2 units/ml after 72 h (Fig. 2).

Reduced APA as well as nonmodified herring sperm DNA did not induce IFN-γ in lymphocytes up to a concentration of 100 μg/ml. Moreover, low molecular fragments of APA consisting of pyrimidine nucleotides, of nucleosides and of pyrimidine bases were likewise ineffective.

*IFN-γ production by lymphocytes after APA incubation together with PHA.* APA given in combination with PHA resulted in a strong synergistic effect on the IFN-γ production. This effect in the combination studies PHA/APA was highest at an APA concentration of 10 μg/ml (Fig. 3). In the case of an additive effect an IFN-γ production of only 398 (366 + 32) ± 60 units/ml would have been expected, while

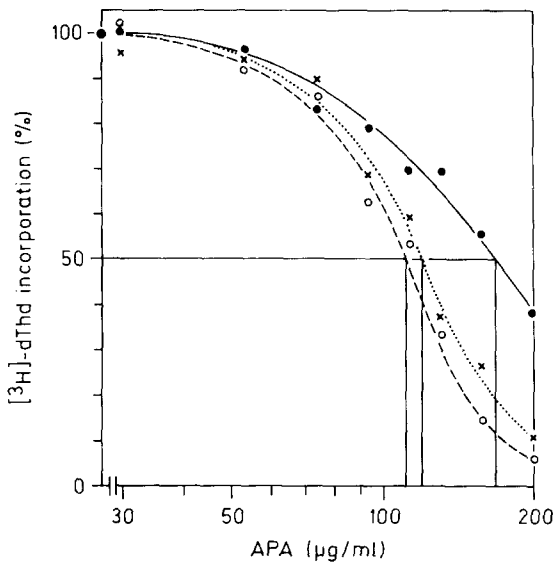


Fig. 1. Influence of APA on [$^3$H]-dThd incorporation into human peripheral blood lymphocytes in the absence (•—•) or the presence of 6.25 μg/ml of PHA (○--○) or 3 μg/ml of PWM (×.....×). Means of six parallel experiments are presented; the S.D. was less than 8%. The values for a 50% inhibition of [$^3$H]-dThd incorporation of the corresponding dose-response curves are given as vertical lines.

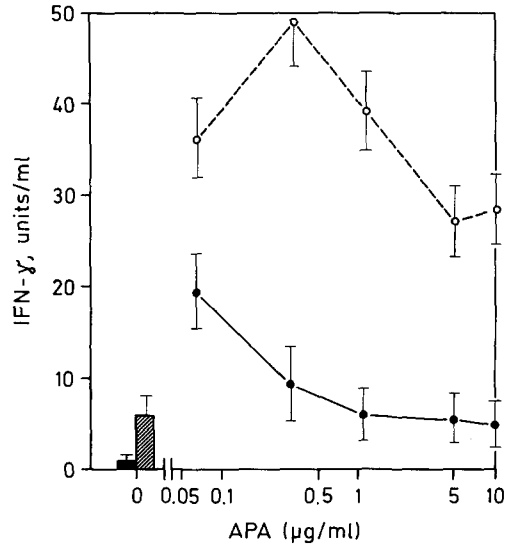


Fig. 2. IFN-γ production by lymphocytes as a function of APA concentration. The cells were incubated in the standard assay for 24 h (•—•) or 72 h (○--○) in the presence of different APA concentrations. Solid column: IFN-γ content in the controls after 24 h; hatched column: idem after 72 h. The supernatants were analyzed for IFN-γ content; the means (± S.D.) of 7 parallel experiments are given

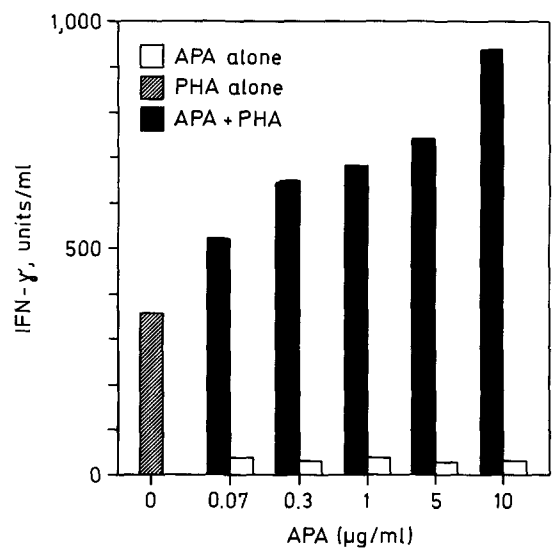


Fig. 3. Synergistic effect of APA together with PHA on the IFN-γ production by lymphocytes. 6.25 μg/ml of PHA were added to the cultures. The incubation period was 72 h. The means of 5 parallel experiments are given; the S.D. was less than 15%.

the experiments revealed at titer of 940 ± 135 units/ml. The synergism was significant at a $P$ value of $< 0.001$.

It should be stressed that the APA concentrations used for the combination experiments were determined not to influence the incorporation rates of [$^3$H]-dThd into lymphocytes.

*Anti-HIV activity of APA*

*In vitro assessment of anti-HIV activity.* Using the introduced H9/HTLV-IIIB cell system, an anti-HIV activity was evaluated at APA concentrations above 10 μg/ml (Fig. 4). At this concentration, the RT activity in the culture supernatant (as a measure for the presence of HIV-1) was reduced to 51%; the expression of HIV-1 proteins p24 and p17 was inhibited by 28% and 32%, respectively; and the number of viable cells per ml increased from 0.52 (untreated) to 0.72 × $10^6$ (APA-treated). At 20 μg/ml of APA, the inhibition of both RT activity and expression of virus proteins p24 and p17 was 71% and 52%, respectively. Increasing the APA concentration to 50 μg/ml resulted in an even more pronounced anti-HIV effect.

*Inhibition of viral mRNA expression.* To establish that APA inhibits viral mRNA expression poly(A)-rich mRNA was extracted from HTLV-IIIB infected H9 cells, grown in the absence or presence of APA, and analyzed by Northern blot procedure. Hybridization was performed with HTLV-IIIB subgenomic cDNA clone

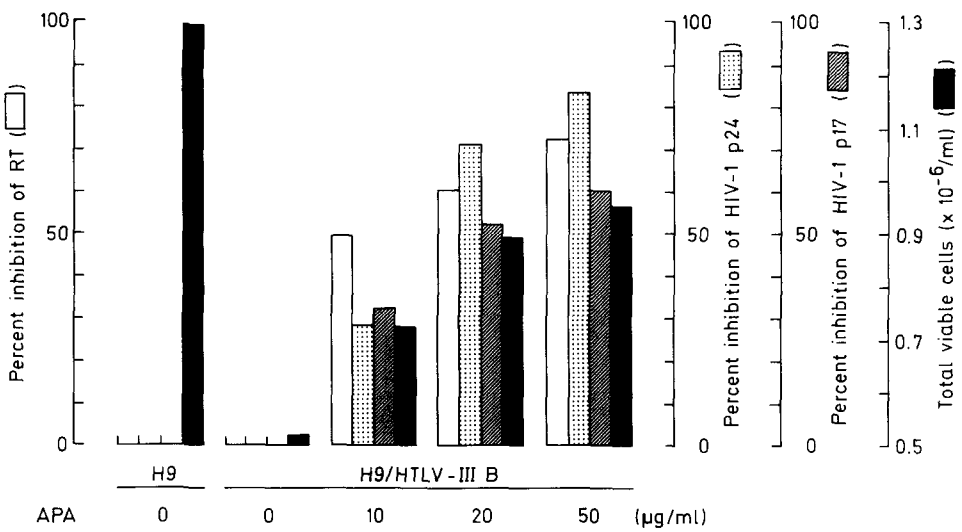


Fig. 4. HIV-1 replication in H9 cells, infected with HTLV-IIIB in the absence or presence of APA. Incubation was performed for 4 days. Then (*a*) the RT activity in the supernatants was determined [the data are given as percent inhibition of RT activity, compared to the infected controls (open bars)]; (*b*) the inhibition of HIV-1 protein p24 (dotted bars) and p17 (hatched bars) expression of infected cells was estimated and (*c*) the number of viable cells was determined (solid bars). As a control, the growth of uninfected H9 is given. The means of six parallel experiments are shown.

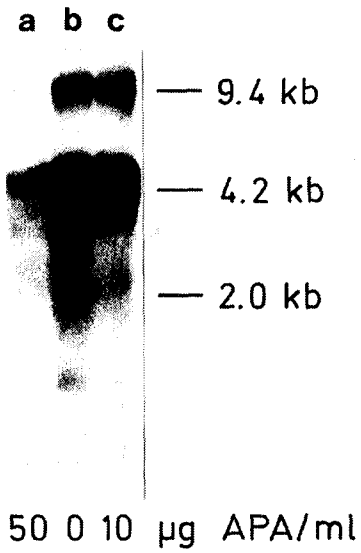


Fig. 5. Inhibition of HIV-1 poly(A)-rich mRNA expression in H9/HTLV-IIIB cells by APA. HTLV-IIIB-infected H9 cells were incubated in the standard assay for 4 days in the presence of 0 (lane b), 10 (lane c) and 50 µg/ml of APA (lane a). Then poly(A)-rich mRNA was isolated and 2 µg RNA were subjected to electrophoresis, transferred to nitrocellulose membrane and hybridized with the subgenomic 3'-*orf*/LTR fragment, consisting also of a major segment of the leader sequence.

containing the 3'-*orf*/LTR fragment and a major segment of the leader sequence. This DNA hybridized to the 9.4 kb (unspliced genomic HIV-1), 4.2 kb (*env*) and 2.0 kb (*tat*-III/3'*orf*; Arya et al., 1985) mRNA from infected cells, not treated with APA (Fig. 5, lane b). Under otherwise identical conditions, the 3'-*orf*/LTR probe reacted strongly with the 9.4 and 5.0 kb and only weakly with the 2.0 kb species in the mRNA fraction from infected cells, treated with 10 µg/ml of APA (Fig. 5, lane c). Infected cells, incubated in the presence of 50 µg/ml of APA contained no detectable 9.4 kb and 2.0 kb mRNA and only very little 4.2 kb mRNA (Fig. 5, lane a). This result demonstrates that APA strongly inhibited HIV mRNA expression.

*Inhibition of HIV-1 RT by APA*

The effect of APA on the three mammalian DNA polymerases α, β and γ as well as HIV-1 RT was tested and in all cases the inhibition was found to be of a pure non-competitive type. This finding is in agreement with earlier data (Müller et al., 1973). The $K_i$ values, which are equivalent to the 50% inhibition values (Dixon and Webb, 1979), are summarized in Table 1. The data show that the $K_i$ values for the mammalian DNA polymerases are in the range of 124 and 271 µg/ml and, hence, 140- to 300-fold higher than the $K_i$ value measured in the RT assay system, using poly(A)·$(dT)_{10}$ as template-primer. Using the template poly(C) in-

TABLE 1

Influence of both nonreduced and reduced APA on the activity of the three mammalian DNA polymerases and the HIV-1 reverse transcriptase

| Enzyme | APA preparation | Template-primer | $K_i$ value | |
|---|---|---|---|---|
| | | | (μg/ml) | (μM) |
| DNA polymerase α | nonreduced | poly(dC)·$(dG)_{12\,18}$ | 271.7 ± 21.3 | 14.3 ± 1.1 |
| DNA polymerase β | nonreduced | poly(dA)·$(dT)_{10}$ | 208.4 ± 15.9 | 11.0 ± 0.8 |
| DNA polymerase γ | nonreduced | poly (A)·$(dT)_{10}$ | 124.1 ± 9.3 | 6.5 ± 0.5 |
| HIV-1 reverse transcriptase | nonreduced | poly (A)·$(dT)_{10}$ | 0.9 ± 0.1 | 0.05 ± 0.005 |
| | reduced | poly (A)·$(dT)_{10}$ | >1000 | >50 |
| | nonreduced | poly (C)·$(dG)_{18}$ | 7.5 ± 0.4 | 0.39 ± 0.02 |
| | reduced | poly (C)·$(dG)_{18}$ | >1000 | >50 |

The inhibition is of the non-competitive type; the $K_i$ value is given both in μg/ml and in μM (with respect to an average MW of APA of $1.9 \times 10^4$ daltons). The means (± S.D.) of 5 experiments are presented.

stead of poly(A), the inhibition of RT was 8-fold lower. Fig. 6 shows the Lineweaver Burk plot from which the kinetic data for the non-competitive inhibition of HIV-1 RT by APA were calculated. They revealed a $K_m$ value of 0.078 ± 0.009 μM [with respect to poly(A)] and a $K_i$ value of 0.05 ± 0.005 μM for nonreduced APA.

In an earlier study we found (Müller et al., 1973) that the inhibitory activity of

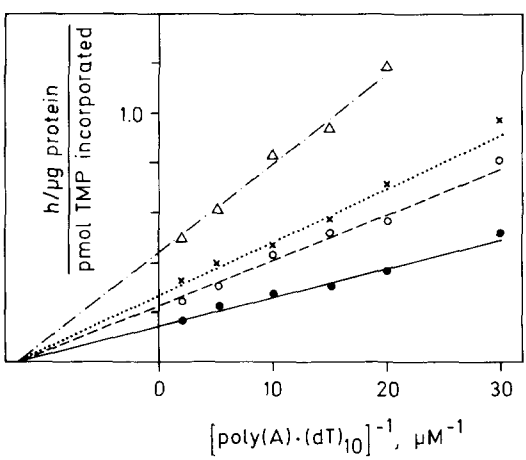


Fig. 6. Kinetics of inhibition (template/primer [poly(A)·$(dT_{10})]^{-1}$ versus 1/v) of HIV-1 RT by APA. Plot according to Lineweaver and Burk (1934). Concentrations of nonreduced APA: control (●—●); 0.02 μM (○--○); 0.04 μM (×.....×) and 0.1 μM (△-.-△). The concentrations of template/primer are expressed in μM with respect to poly(A); oligo$(dT)_{10}$ at 1/6 concentration of poly(A). The $K_m$ was 0.078 ± 0.009 μM and the $K_i$ was 0.05 ± 0.005 μM.

APA on mammalian DNA polymerases was almost completely abolished if the aldehyde groups present in the modified DNA were reduced. This finding can now be extended for HIV-1 RT; the $K_i$ value determined with a reduced APA preparation was higher than 1000 μg/ml (Table 1).

## Discussion

It is generally assumed that polydeoxyribonucleotides, DNA or DNA-RNA hybrids are not inducers of IFN (Baron et al., 1979). Now we describe that the chemically modified DNA, APA, is an inducer of IFN-γ in the human blood lymphocyte system in vitro. This effect is caused at APA concentrations (0.05–1 μg/ml) which are 300-fold lower than those required for inhibition of cell growth (as determined by the incorporation of [$^3$H]-dThd into DNA). At the optimal concentration (0.4 μg/ml) 50 units/ml were measured in the culture supernatant after an incubation period of 72 h. This titer has previously been reported (*i*) to establish an antiviral state (Springfellow, 1985) and (*ii*) to inhibit HIV-1 growth in CEM cells by 50% (Montefiori and Mitchell, 1987). Equally important is the finding that APA at concentrations between 0.07 and 10 μg/ml potentiates the mitogen[PHA]-induced IFN-γ formation. This result raises the question as to whether APA enhances IFN-γ production in a functionally similar manner as naturally occurring stimuli, e.g. IL-2 (Verelizier, 1985). However, the molecular mechanism by which APA induces IFN-γ is apparently different from IL-2. Unpublished data show that APA does not interact with the IL-2 receptor but enters the cell and binds to nuclear structural elements. Moreover APA inhibits the 2′,3′-exoribonuclease (Schröder et al., 1980) activity and hence prolongs the antiviral function of pppA(2′p5′A)$_n$ (unpublished). Since deficient IL-2 secretion is thought to be one reason for the impaired production of IFN-γ by AIDS T cells (Murray et al., 1984), APA is a candidate drug substituting IL-2 deficiency in AIDS patients. Applying the same IFN assay procedure, APA was found not to induce IFN-α or IFN-β in the human blood lymphocyte system in vitro (to be published).

The second newly discovered feature of APA is its anti-HIV activity in the H9/HTLV-IIIB system. At an APA concentration of 10 μg/ml the HIV-1 production (measured on the basis of RT activity) was inhibited by approximately 50%. The inhibition of virus growth was accompanied by a cytoprotective effect and by an inhibition of expression of the HIV-1 p17 and p24 *gag* proteins; these effects were most pronounced at APA concentrations of 20 and 50 μg/ml. In order to test whether APA also blocks viral mRNA expression in target H9 cells exposed to the virus and cultured with or without APA, total poly(A)-rich mRNA was isolated from the respective cultures. After electrophoretic separation the RNA was blot transferred and hybridized with HIV-DNA from the 3′-*orf*/LTR region, including the polyadenylation site. The results revealed a strong decrease in the amount of viral poly(A)-rich mRNA in H9 cells treated with APA. Taken together, these data show that APA blocks virus growth at the level of transcription, or even earlier in the life cycle of the virus.

To elucidate the mode of action of APA as anti-HIV agent we determined its inhibitory ability on HIV-1 RT. We found that this enzyme is 140- to 300-fold more susceptible to inhibition by APA than the cellular DNA polymerases α, β and γ. The inhibition is of the non-competitive type and only marginally depends on the template-primer used.

From the reported data we conclude that APA displays in vitro two potentially useful effects; (*i*) induction of IFN-γ and (*ii*) inhibition of HIV-1 replication. In addition, APA has previously been found to inhibit growth of tumor cells both in vitro (Müller et al., 1973) and in vivo (Goldenberg and Heicke, 1971).

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