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A BIOLOGICAL RESPONSE MODIFIER, PSK, INHIBITS REVERSE TRANSCRIPTASE IN VITRO

[1]Kunitaka Hirose, [1]Michinori Hakozaki, [1]Junji Kakuchi, [1]Kenichi Matsunaga, [1]Chikao Yoshikumi, [1]Masaaki Takahashi, [2]Tadafumi S. Tochikura and [2]Naoki Yamamoto

[1]Biomedical Research Laboratory, Kureha Chemical Industry Co., Ltd., 3-26-2 Hyakunin-cho, Shinjuku-ku, Tokyo 160, Japan

[2]Dept. of Virology and Parasitology, Yamaguchi University School of Medicine, Ube, Yamaguchi 755, Japan

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We found that PSK has an antiviral effect on human immunodeficiency virus (HIV) in vitro. One of the mechanisms of this effect is attributable to the inhibition of binding of HIV with lymphocytes. Here, we found that PSK inhibits reverse transcriptase in a non-competitive way in vitro. Such inhibition may be important in its anti-HIV effect as well as its inhibitory effect on the binding of HIV with lymphocytes.

The polysaccharide preparation termed PSK is isolated from Corioulus versicolor by hot water extraction of mycelia, followed by saturated ammonium sulfate precipitation, and desalting by dialysis against water (1).

Many reports describe the antitumor effect of PSK, which is now available for clinical use in Japan (2).

This antitumor effect of PSK is generally thought to be mediated through the host immune system as a biological response modifier (BRM). PSK also inhibits the growth of HIV in vitro (3).

Abbreviations: PSK, Protein-bound polysaccharide Kureha; ddCTP, 2',3'-dideoxycytidine 5'-triphosphate; ddNTP, 2',3'-dideoxynucleoside 5'-triphosphate; [iO^{2}A)n]^{46}, poly(2-iodoadenylic acid); AIDS, Acquired immune deficiency syndrome.

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One of the mechanisms of this effect is the inhibition of HIV from binding with lymphocytes (3).

We found that PSK also inhibits reverse transcriptase in vitro. Here we describe the inhibition by PSK of reverse transcriptase from the enzymological viewpoints.

MATERIALS AND METHODS

Enzyme Purified reverse transcriptase (RT) of avian myeloblastosis virus (AMV) was purchased from Boehringer Mannheim Yamanouchi Co., Ltd., Tokyo, Japan.

Template/Primer Total mRNA was isolated from the livers of female Wistar rats according to the method described by Maniatis (4). It was mixed with oligo(dT)$_{12-18}$ (P.L. Biochemicals, Inc.) to give a stock solution containing 1mg/ml of mRNA and 0.2mg/ml of oligo(dT)$_{12-18}$.

Drug PSK (Kureha Chemical Ind. Co., Ltd.) was dissolved in sterile distilled water and stored at -20°C until use.

4dNTP dATP, dGTP, dCTP and dTTP were purchased from P.L. Biochemicals, Inc. and [α-^{32}p]dCTP(800Ci/mmol) was purchased from Amersham-Japan, Inc..

Standard assay procedure The standard assay contained 50 mM Tris-HCl (pH 8.3), 50 mM KCl, 8 mM $MgCl_2$, 8mM DTT, 40 µg/ml mRNA, 8 µg/ml oligo(dT)$_{12-18}$, 40 µM 4dNTP containing 5 µCi of [α-^{32}p] dCTP and 40 units/ml RT in a volume of 25 µl. Incubation was carried out at 37°C for 1.0 hr. The incorporation of [α-^{32}p]dCTP was measured by a method described previously (5).

RESULTS

First, 25-200 µg/ml of PSK was added to the standard assay solution to examine the inhibition of RT activity at different concentration of PSK. PSK inhibited RT in a dose-dependent way, and caused 50% inhibition (IC_{50}) at the concentration of about 30 µg/ml (Fig. 1). Under this reaction condition, the IC_{50} of ddCTP(6), which is known to have RT inhibitory activity as a substrate analogue was 0.2 µg/ml (data not shown).

We also studied whether the inhibition by PSK was changed by varying the concentrations of RT, 4dNTP and template/primer in the standard assay solution. The results summarized in Table 1 demonstrated that the inhibitory activity of PSK reduced with an inverse relationship with increasing the concentration of RT. However, no effect was observed by varying the concentration of

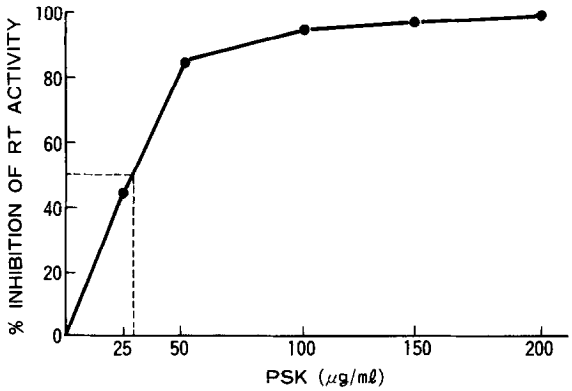

Figure 1. Inhibitory effect of PSK on the RT activity of AMV. Varied concentrations of PSK was mixed with standard RT assay solution. The standard assay procedure is described in MATERIALS AND METHODS. The dashed line represents the concentration of PSK that inhibits 50% of RT activity (IC_{50}).

4dNTP and template/primer. These results motivated us to conduct a detailed kinetic analysis. The Lineweaver-Burk plots showed the effect of varying the concentrations of 4dNTP(Fig. 2A) and

Table 1. Effect of PSK on AMV RT activity at various concentrations of template/primer, subsrate and enzyme

a) Effect of the concentration of RT

RT(Units/mℓ)	% Inhibition
800	20
280	25
160	64
40	80
20	89

b) Effect of the concentration of substrate

4dNTP (μM)	% Inhibition
80	85
40	78
20	80
4	82
2	78

c) Effect of the concentration of template/primer

mRNA(μg/mℓ)	$Oligo(dT)_{12-18}$(μg/mℓ)	% Inhibition
40	8	87
10	2	82
4	0.8	85

PSK was used at a concentration of 50μg/mℓ. The standard assay procedure is described in MATERIALS AND METHODS.

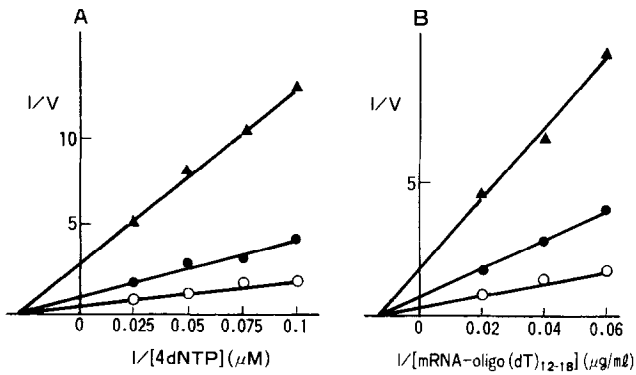

Figure 2. Lineweaver-Burk plots of AMV RT activity inhibited by PSK as a function of substrate and template/primer concentrations. The panels correspond to experiments in which the concentrations of 4dNTP(A) and mRNA-oligo$(dT)_{12-18}$(B) were varied. The standard assay procedure is described in MATERIALS AND METHODS. Values of V correspond to pmol of dCMP incorporated per 10 min. assay.
o: no inhibitor, ●: PSK 20μg/mℓ, ▲: PSK 50μg/mℓ

template/primer(Fig. 2B) on RT activity in the presence of 20 or 50 μg/ml of PSK. The Vmax decreased in the presence of PSK, but the Km for 4dNTP or template/primer was unchanged. These results indicate that PSK inhibited RT activity in a non-competitive way.

DISCUSSION

PSK was found to be a powerful inhibitor of AMV reverse transcriptase. The inhibition by PSK was not affected by varying concentrations of the substrate and template/primer, but significantly reduced when the concentration of the enzyme increased. Lineweaver-Burk plots showed that the inhibition of RT activity by PSK was non-competitive with substrate and template/primer. These results suggest that PSK acts directly on the RT molecule. Thus, PSK seems to be a specific enzyme inhibitor of RT. RT of retroviruses plays a critical role in the integration of the viral RNA genome into host cell DNA (7). A specific inhibitor of the enzyme might be useful as an agent against infection by HIV, the causative virus of AIDS.

Many RT inhibitors have therefore been developed. In terms of their mode of action, they were divided into the following classes: (i) enzyme-binding compounds such as suramin (8), (ii) substrate analogues such as ddNTP (6), (iii) template/primer-binding compounds such as actinomycin D and daunomycin, and (iv) template analogues such as $[(io^2A)n]^{4,6}$ (9).

This study suggests that PSK is an inhibitor belonging to the enzyme-binding class of compounds. PSK has been shown to exert a beneficial therapeutic effect against various tumor both as a single agent (10) and in combination with other therapies (11, 12) in the clinical field. Though the antitumor mechanism of PSK is not fully understood, host-mediated activity has been claimed to play a significant role. In fact, abundant evidence has been presented that the administration of PSK to tumor-bearing rodents inhibited tumor growth and also modulated immunoresponses such as the induction of killer T cells (13, 14), activation of macrophages (14, 15) and natural killer cells (16), and enhancement of capacity to produce antibody (14, 17). The administration of PSK, which activates the immune system, might improve the immune condition of AIDS patients and help prevent the risk of opportunistic infections and kaposi's sarcoma.

PSK can inhibit both the binding of HIV with lymphocytes (3) and RT activity as well as possibly activating host immune function, so it may be useful for the prevention of the risk of HIV infection and treatment of AIDS.

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