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POLYSACCHAROPEPTIDE FROM *CORIOLUS VERSICOLOR* HAS POTENTIAL FOR USE AGAINST HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 INFECTION

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Abstract. Polysaccharopeptide (PSP) isolated from the edible mushroom *Coriolus versicolor* was tested for its potential as an anti-human immunodeficiency virus type 1 (HIV-1) compound in a series of in vitro assays. It demonstrated inhibition of the interaction between HIV-1 gp120 and immobilized CD4 receptor ($IC_{50} = 150\mu\text{g/ml}$), potent inhibition of recombinant HIV-1 reverse transcriptase ($IC_{50} = 6.25\mu\text{g/ml}$), and inhibited a glycohydrolase enzyme associated with viral glycosylation. These properties, coupled with its high solubility in water, heat-stability and low cytotoxicity, make it a useful compound for further studies on its possible use as an anti-viral agent in vivo. © 1997 Elsevier Science Inc.

Key Words: polysaccharopeptide, *Coriolus versicolor*, reverse transcriptase, glycohydrolase, HIV-1

Introduction

Polysaccharopeptide (PSP) is a substance produced by the edible mushroom *Coriolus versicolor* (1). It has been claimed to possess antitumor activity and has been shown to activate the transcription of tumour necrosis factor gene in mouse peritoneal macrophages, indicating that it has an immunomodulatory effect (1). PSP was also demonstrated to have a very low cytotoxicity (1). PSP is a 100kDa molecule and analysis indicates that the polysaccharide component is composed of 74.6% glucose, 4.8% xylose, 2.7% galactose, 2.4% fructose and 1.5% mannose. The amino acid composition is 0.58% Glu, 0.4% Asp, 0.32% Ser, 0.26% Ala, 0.26% Gly, 0.24% Leu, 0.23% Lys, 0.23% Thr, 0.22% Ile, 0.18% Arg, 0.18% Val, 0.17% Trp, 0.15% Phe, 0.15% Tyr, 0.1% Pro, 0.09% Cys, 0.07% His and 0.04% Met (1). The immunomodulatory activity of PSP has been demonstrated but its anti-viral properties, particularly anti-HIV-1, have not been investigated. Here we test PSP for inhibitory activity in an HIV-1 gp120/CD4 receptor EIA, an in vitro recombinant HIV-1 reverse transcriptase assay and against a variety of glycohydrolase enzymes.

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Methods

Coriolus versicolor (strain Cov-1) is cultivated as a deep-layer submerged culture on a medium of 78% sawdust, 18% bran, 1% glucose and 1% calcium sulphate at 20-25°C. Cultivation continues for 3-4 weeks. Harvested mycelia are extracted with hot water and PSP is precipitated with 80% (v/v) ethanol. The precipitate is washed with acetone, anhydrous ethanol and ether (2). PSP isolated in this manner is a light-brown friable powder. PSP was obtained from Landford PSP Ltd. (Hong Kong). It is identical to the commercial product "Essence of mushroom (Yun Zhi)" sold as a health food supplement. The product is readily available in Hong Kong from pharmacies and health food shops. PSP was dissolved in distilled water to 5mg/ml and sterilized by passing through a 0.22µm filter (Millipore, U.S.A.). The PSP preparation was pure according to the manufacturer as judged by TLC, gel filtration and SDS-PAGE.

The non-radioactive recombinant HIV-1 reverse transcriptase ELISA kit was obtained from Boehringer Mannheim, Germany. The screening assay for inhibitors was performed as described in the protocol included with the kit, except that each sample well contained 2ng recombinant reverse transcriptase. The HIV-1 gp120/immobilized CD4 receptor EIA was obtained from NEN Dupont (Boston, MA, U.S.A.). The screening test for inhibitors was performed as described in the protocol included with the kit. Each sample well contained 1ng standard HIV-1 gp120. The enzymes α -glucosidase (E.C. 3.2.1.20), β -glucosidase (E.C. 3.2.1.21) and β -glucuronidase (E.C. 3.2.1.31) were from Sigma Chemical Co. (St.Louis, MO, U.S.A.). The substrates *p*-nitrophenyl- α -D-glucopyranose, *p*-nitrophenyl- β -D-glucopyranose and *p*-nitrophenyl- β -D-glucuronide were from Sigma Chemical Co. The glycohydrolase assay was performed in 96 well microtiter plates as described previously (3). The assays were performed in duplicate. The results obtained were within $\pm 5\%$ of the mean. All other reagents were the best commercially available.

Results

The effect of PSP on the potential interaction of HIV-1 and its target cell was investigated using an HIV-1 gp120/immobilized CD4 receptor EIA. Aqueous extracts of PSP were tested in a range of concentrations from 0-0.2mg/ml. PSP was an inhibitor of the interaction. The IC_{50} was 0.15mg/ml. This corresponds to a concentration of 1.5µM based on a molecular mass of 100kDa.

PSP was tested to determine its inhibitory effect on recombinant HIV-1 reverse transcriptase. Aqueous extracts of PSP were tested in the range 0-5mg/ml. The results are shown in Figure 1. The IC_{50} for the inhibition was 6.25µg/ml. This corresponds to a concentration of 62.5nM. PSP appears to be as potent as the reverse transcriptase inhibitory compounds isolated from natural products that have been described previously e.g. nitidine chloride and fagoronine chloride (4). The heat stability of the PSP preparation was determined. A 1mg/ml solution of PSP was heated to 100°C for 5 minutes. The heating had no effect on its ability to inhibit reverse transcriptase (Table 1).

PSP was tested for its ability to inhibit a number of glycohydrolase enzymes associated with viral protein processing. The effect of heating PSP on its ability to inhibit the glycohydrolase enzymes was also examined. The combined results are shown in Table 2. The inhibitory effect on β -glucuronidase was examined in further detail. Examination of Lineweaver-Burk plots using different concentrations of PSP revealed that the inhibition was of the "mixed" type (5), with effects on both the maximum velocity (V_{max}) and K_M (Figure 2). The K_i was 46.4 µg/ml.

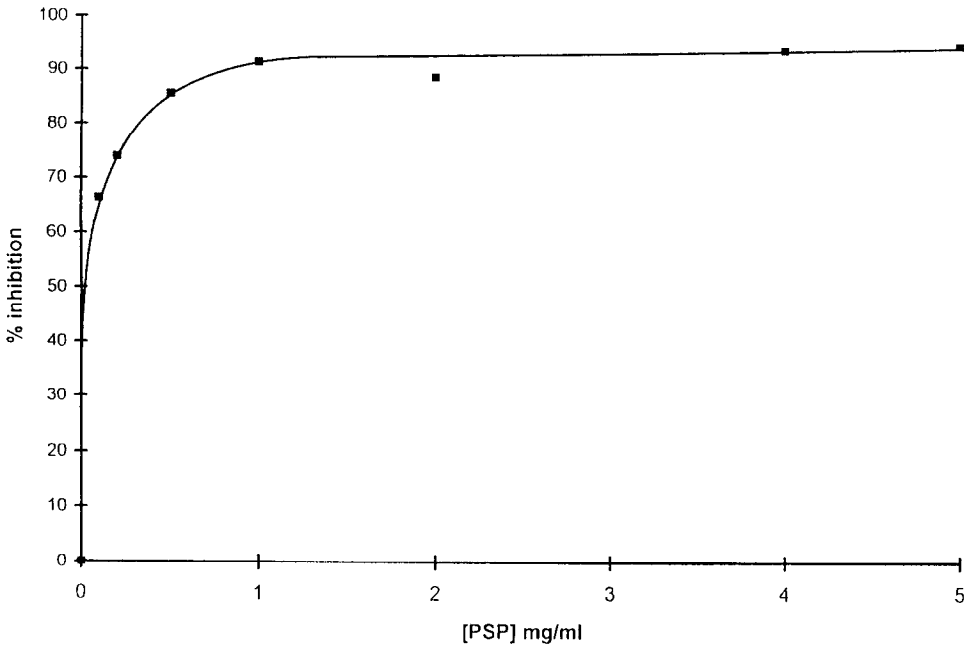


Fig. 1

Inhibition of HIV-1 reverse transcriptase by an aqueous solution of PSP. Reverse transcriptase activity was determined as described in Methods. Enzyme activity is normalized with respect to an uninhibited control sample.

TABLE 1.

Effect of PSP on Recombinant HIV-1 Reverse Transcriptase

Dose (μ g/ml)	% inhibition	
	Control	Heated
25	69.0	70.5
50	76.8	78.9
100	83.4	86.8

Values are means of duplicate measurements that did not vary by more than 3%.

TABLE 2

Effect of PSP on Glycohydrolase Enzymes

Treatment	% inhibition*		
	α -glucosidase	β -glucosidase	β -glucuronidase
Control	0	15.1	75.4
Heated	0	15.4	76.3

* PSP present at 0.2mg/ml in each case. Values are means of duplicate measurements that did not vary by more than 5%.

Discussion

PSP has the potential to be a useful agent in the fight against viral infections, especially HIV-1. PSP has previously been shown to have low cytotoxicity to mammalian cells and is highly soluble in water (1). This report is the first to describe the potential anti-viral properties of PSP. The inhibition of the interaction between HIV-1 gp120 and immobilized CD4 receptor in vitro is very potent ($IC_{50} = 1.5\mu M$) and demonstrates the potential of PSP to interfere with the binding of the HIV-1 virus to its cellular target in vivo. The observed inhibition is likely to be mediated by the extensive carbohydrate moiety of PSP binding to HIV-1 gp120 and affecting the binding of virus to the CD4 receptor, or vice versa. A sulphated polysaccharide, with a molecular weight of 10kDa, isolated from the Chinese medicinal herb *Prunella vulgaris* inhibited the interaction of HIV-1 gp120 with CD4 both in vitro and in vivo (6,7). Further studies are necessary to elucidate the precise mode of binding of PSP

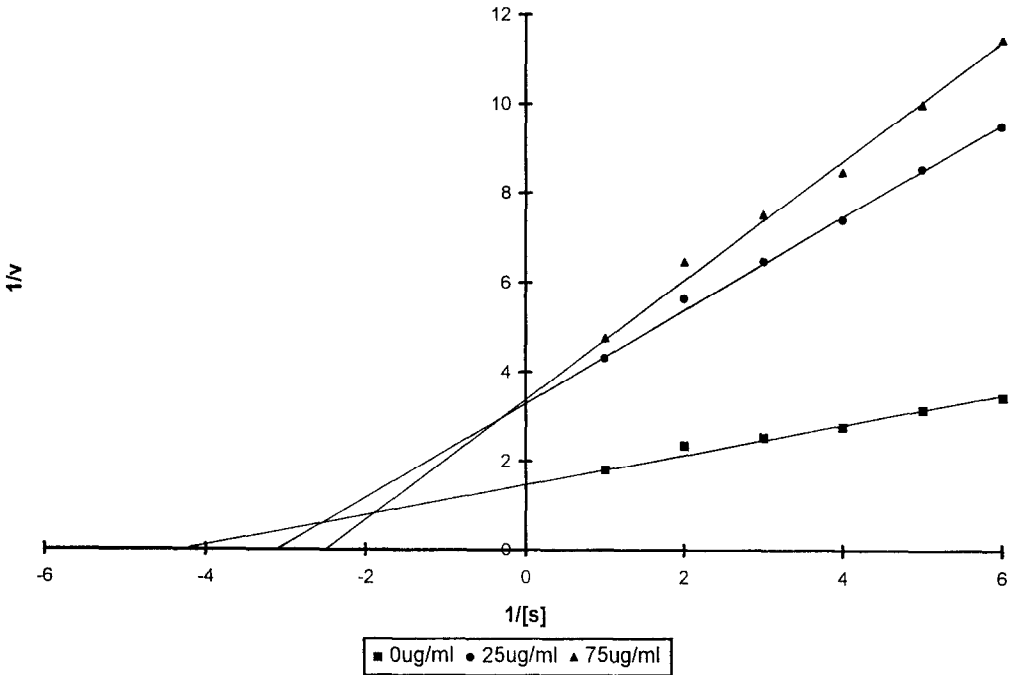


Fig. 2

Analysis of inhibition of β -glucuronidase by an aqueous solution of PSP.

The enzyme was incubated with PSP as indicated and assayed as described in Methods. The K_i was found to be $46.4\mu g/ml$.

PSP also has a very potent inhibitory effect against HIV-1 reverse transcriptase in vitro (Fig. 1). The IC_{50} of $6.25\mu g/ml$ makes PSP one of the few natural products so far isolated to have specific reverse transcriptase inhibitory properties. It is common practice in Asia for natural product-derived remedies to be taken as hot water decoctions. The inhibitory effect of PSP on reverse transcriptase is not affected by boiling indicating the stability of the molecule (Table 1). The size of the molecule (100kDa) may preclude the possibility that the PSP binds to the active site of reverse transcriptase.

Glycohydrolytic enzymes are found in the Golgi complex and are associated with viral protein processing (8). α -Glucosidase in particular has been found to be involved in HIV-1 gp120 processing

(8). Although PSP was not found to inhibit α -glucosidase viral protein glycosylation could be disrupted by its effects on other intracellular glycohydrolase enzymes. The "mixed" type nature of the inhibition of β -glucuronidase by PSP indicates that the compound is not acting as a substrate mimic but acts by binding to sites on the enzyme distinct from the catalytic active site (5). The inhibition of the glycohydrolase enzymes was also unaffected by boiling the PSP (Table 2). MAR-10, a substituted polysaccharide isolated from *Hyssop officinalis* was able to inhibit HIV-1 replication in vitro. It was also found to be a broad spectrum glycohydrolase inhibitor (9). A number of other aqueous extracts of natural products have been found to inhibit the same glycohydrolase enzymes described here as well as other aspects of the HIV-1 life cycle (3).

PSP is certainly as potent as other natural products that have been demonstrated to possess anti-HIV-1 properties e.g. *Viola yedoensis* extract (10,11) and glycyrrhizin (12). All of the activities described here for PSP need to be confirmed with in vitro studies using whole cell and virus preparations and with in vivo studies. The multivalent manner in which PSP acts in vitro to inhibit several different facets of the HIV life-cycle should make it an ideal candidate for more detailed in vivo studies. The high water solubility, heat-stability and demonstrated non-cytotoxic nature of this compound make it a useful lead candidate for such studies.

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