The HPLC Separation and Bioactivity Evaluation of the Polysaccharopeptide (PSP) of *Coriolus* (*Trametes*) *versicolor* Fermentative Mycelia

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Polysaccharopeptide (PSP, commercial name I'm-Yunity™) is isolated from the deep-layer cultivated mycelia of *Coriolus versicolor* Cov-1™ strain, using patented manufacturing processes. It is a covalently linked protein/polysaccharide complex with a molecular size reported as 100 kD. The polysaccharide moiety consists of 56.1% β-(1, 3) glycan. Its peptide moiety contains 18 kinds of amino acids in which

the glutamic acid and aspartic acid are the most abundant.

The pharmacological study and clinical trials have proved that PSP possesses immunomodulatory effects that can benefit cancer patients by improving their immunity and life quality. It also can antagonize the side-effects caused by radio- or chemotherapy.

Previous studies have shown that PSP has several fractions. Electrophoresis results showed that PSP had three bands, and similar results were obtained using column chromatography. Yang MMP and colleagues separated PSP into two fractions by using reverse phase HPLC and detected the first eluant possessing direct antitumor activity. However, the contributions of all PSP fractions to its bioactivities are still not well understood. Therefore, in this study, we applied an anion ion-exchange HPLC method to separate PSP and evaluated the bioactivity of its fractions.

The commercial PSP product was dissolved in 95 °C water and centrifuged to remove auxiliary materials and precipitants. It was further desalted and freeze-dried prior to HPLC separation. The freeze-dried powder was dissolved in an initial buffer and injected into a preparative anion ion-exchange HPLC column. The column was eluted with initial buffer following an increased NaCl gradient. After that, PSP was separated into four fractions: Fr1, without a negative charge; Fr2, with a weak negative charge; Fr3, with a moderate negative charge; and Fr4 with a strong negative charge. Chemical analysis reveals that the polysaccharide content of Fr2 is the highest (92.5%), while the Fr4 is the lowest (35.2%). However, the β-(1, 3) glucan contents in polysaccharides are reversed, and the Fr4 has the highest content (51.0%), while Fr1 and Fr2 have almost no β-(1, 3) glucan detected. Fr1 and Fr2 also have roughly half-protein contents compared to Fr4 and Fr3, and the highest content is in Fr3 (22.6%).

The mouse splenocyte proliferation assay reveals that the Fr4 and Fr3 have stronger immunostimulatory effects than Fr2 and Fr1. The stimulatory index of Fr4, Fr3, Fr2, and Fr1 are 1.29, 1.27, 1.12, and 1.08, respectively. Fr3 and Fr4 also elevate mouse splenocyte $CD4^+/CD8^+$ ratio, while Fr1 and 2 retained or slightly decreased compared to unfractionized PSP. All four fractions strongly stimulate the production of IL-6 and TNF-α from human PBMN cells. The IL-6 production was increased up to around 8.1 fold by Fr 2 and 4, and TNF-α level was even heightened to 12.7 folds by Fr 2 and Fr 3.

In conclusion, PSP probably contains several compounds, and these compounds can be fractionized using an anion ion-exchange HPLC method. Different fractions showed diverse pharmacological activities as well as different physical and chemical properties. To understand the relation of PSP fractions to their bioactivities, it may help us to set up specific quality control methods to monitor and to improve PSP quality and to develop the product with higher efficacy.

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