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THE BIOACTIVITY OF
PHOSVITIN PHOSPHOPEPTIDES
A GENERAL REVIEW OF THE SCIENTIFIC LITERATURE

ALEX HOLDCROFT, BSC., MM
ECOVATEC SOLUTIONS INC.
31231 Wheel Ave., Abbotsford, B.C., CANADA, V2T6H1

Executive Summary

Ecovatec's revolutionary technology has unlocked the amazing potential of phosvitin phosphopeptides (PPP). Ecovatec is the only known commercial producer of phosvitin phosphopeptides in the world. Phosvitin is isolated using EcovaPure™ technology from commercial egg yolk and it is prepared into its bioactive peptides, called PPP. PPP has been characterized by many scientific papers, and the literature consensus is that it has huge potential as a bioactive molecule. Bioactivity is based on the ability of a compound to be “able to exert a biological effect at a physiological level, and it must be measurable and able to provide health benefits.”¹ The scientific community has done extensive research on the uses of phosvitin and PPP but have not been able to perform clinical trials due to the difficulty and expense of isolating phosvitin. *Samaraweera, et al (2011)*² and *Ren, et al (2015)*⁴ both note that the potential use of phosvitin (and PPP) as a functional ingredient is only hampered by lack of a scalable method of extraction. **Ecovatec Solutions Inc has eliminated this barrier for researchers and general consumers alike.**

Background

Phosvitin is the most highly phosphorylated protein found in nature and is derived from the egg yolks of vertebrates². Proteins are made up of peptides, which are short chains of amino acids linked by peptide bonds. Bioactive peptides have shown the most promising potentials as therapeutic or health promoting agents².

Serine is the major amino acid present in phosvitin (50%)¹, and almost all the serine residues are phosphorylated. This phosphorylation gives phosvitin its unique properties, including its strong **antioxidant, antiviral, antibacterial, anticancer, and metal chelating** properties.¹⁻⁴ While full-protein phosvitin exhibits some of these properties, it is only when it is partially de-phosphorylated and hydrolyzed into smaller peptides that it fully realizes its potential. EcovaPure™ processing makes these changes to phosvitin while preserving the full bioactivity of the peptides.

The Properties of Phosvitin Phosphopeptides (PPP)

Antioxidant; Nutraceutical and Food Industry Applications

According to Lobo, et al (2010)⁶, when free radicals such as oxygen and nitrogen, which are generated by the body in its daily functions and systems, build up and are out of balance with available “antioxidants” the body undergoes oxidative stress. Oxidative stress is known to be associated with many diseases⁵⁻⁷. An antioxidant is any molecule stable enough to bind to a free-radical and neutralize it, thereby delaying or inhibiting cellular damage.⁶⁻⁸

Some of the oxidative stress related disorders include: **cardiovascular diseases, carcinogenesis, ageing, neurological disorders** (Alzheimer's disease, Parkinson's disease, muscular dystrophy), **inflammatory diseases** (such as arthritis), **diabetes, and many others**.⁶⁻⁸ Oxidative stress can also change the structure of the fats and proteins in the body thereby changing their functions.⁶

Many researchers have studied the properties of PPP as an antioxidant and have agreed that they have great potential to be used both internally as a supplement in the body to promote health and reduce oxidative stress and used as a food ingredient to improve the quality and shelf life of food products⁶⁻¹³.

Increasing Calcium Bioavailability; Nutraceutical Applications

PPP can treat for **low bone density or calcium deficiency** related health concerns as it can increase the **bioavailability of calcium** in the intestines¹⁵⁻¹⁹. For example, osteoporosis, osteomalacia, and rickets are bone disorders linked to calcium metabolism disorders. Calcium has also been suggested to have a role in preventing **colorectal cancer, cardiovascular disease, high blood pressure, preeclampsia, and in promoting weight management**¹⁴. It can also increase the reaction rate of the formation of a bone mineral precursor that can be easily absorbed in the intestines to increase bone density and to create bone fillers and food supplements.¹⁵⁻¹⁶

Inhibiting Melanogenesis; Cosmetic Applications

Melanin is synthesized in melanocytes in the deepest level of the skin to protect the skin from ultraviolet radiation (UV) damage⁵. When large amounts of melanin are produced and accumulate in cells, aesthetic problems such as hyperpigmentation (melasma) and age spots can be developed. **These cosmetic issues can also be associated with an increased risk for skin cancer.** Therefore, it is important for both medical and cosmetic uses to regulate or inhibit melanogenesis.^{5,20}

Researchers have found that, *in vitro*, melanoma cells grown in the presence of PPP have decreased cell viability due to the PPP inhibiting the pathway that promotes melanin production⁵. Further research is expected to be done on the use of PPP in skin creams and lotions to reduce the appearance of age spots, freckles, and moles.

Technology Solutions for Healthy Living

Antibacterial, Toxin Neutralizing, Antiviral; Pharmaceutical Applications

Antimicrobial peptides are being investigated by researchers, as they can kill microorganisms directly by disrupting their cell functions in multiple pathways, making drug resistance unlikely²¹. *Li et al* (2016)²¹ have found that a phosvitin peptide which was specifically tested on **multi-drug resistant bacteria** and found to affect multiple cellular processes to lead to **bacteria cell death**. They note its promising therapeutic potential.

Another microorganism that is associated with high patient mortality is endotoxin (lipopolysaccharide, LPS). This causes inflammation and sepsis in patients and is part of the cell wall of some bacteria²². There is no current therapy to protect from endotoxin-mediated tissue damage and organ failure. Current treatment methods still have **over 50% mortality**.²² *Ma et al* (2012)²² note that antimicrobial peptides are the ideal candidates for future therapies as they have the potential to bind endotoxin and prevent it from binding to body cells that cause the inflammation. When compared to an antibiotic called PMB, **phosvitin peptides were not only effective to kill the bacteria, but it showed no cell toxicity to human cells**. The other drug was slightly more effective, but it is severely toxic to human body cells. The researchers concluded that hen-derived phosvitin (that is, PPP) is “an endotoxin neutralizing agent with therapeutic potential in clinical treatment of LPS-induced sepsis.” *Hu et al* (2013)²³ further corroborated these findings.

Moon et al (2014)²⁴ investigated the effects of phosvitin peptides on various **cancer cell lines in vitro**. For **cervical cancer, PPP was 95% effective** at killing the cancer cells. PPP slowed the growth of breast cancer cells and inhibited the growth of stomach cancer cells by 94%. The effects of phosvitin peptides were most pronounced in killing liver cancer cells.

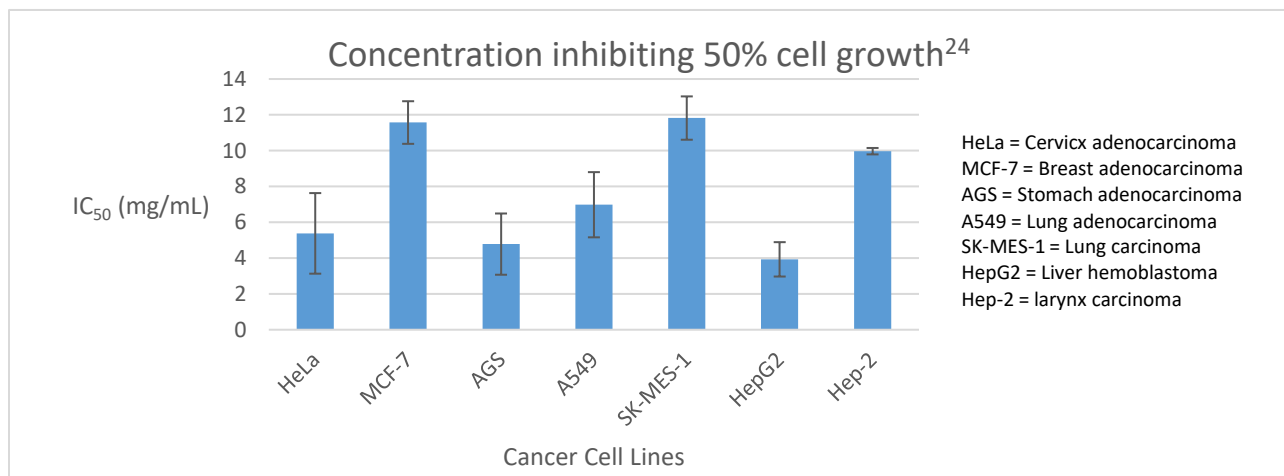


Figure 1. Concentration of phosvitin peptides inhibiting 50% of human cancer cell growth in vitro due to cytotoxic effects. Mg/mL refers to the milligrams of phosvitin present per mL of cell culture. **Lower concentrations** indicate that phosvitin is **more effective** at inhibiting the cancer growth. The most effective cytotoxic effects are seen in cervical adenocarcinoma, stomach adenocarcinoma, lung adenocarcinoma, and liver hemoblastoma.

The **antiviral properties** of phosvitin peptides are also being investigated. *Sun et al.* (2013)²⁵ showed that a peptide isolated from phosvitin was able to protect against a virus that affects many marine and freshwater fishes *in vitro*. While this cannot be extrapolated to human applications yet, it does illustrate that many antimicrobial peptides might also act as antiviral peptides and phosvitin should be researched as a valid antiviral pharmaceutical.

Commercial Applications

Ecovatec’s phosvitin phosphopeptides can be bought in bulk and can be used in a variety of applications:

- Package into pills as a **nutraceutical** and health-promoting supplement
- Include in **food processing facilities** as a natural antioxidant and shelf-life extender
- Use as an ingredient in **skin creams and lotions** to prevent skin cancer, ageing, and hyperpigmentation
- Research **pharmaceutical therapies** and perform clinical trials to approve the use of PPP as an antibiotic, cytotoxic, or antiviral drug.

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