A cost-effective, improved preparation of purified *Paenbacillus polymyxa* neutral protease (Dispase™ equivalent enzyme)

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Background

Dispase™ (trademark of Godo-Sushei) is a P. polymyxa neutral protease (PPNP) initially manufactured as a enzyme to replace trypsin for isolating cells from tissue or recovering adherent cells from tissue culture vessels. For many cells lines, PPNP was as effective or superior to trypsin in recovering adherent cells after *in vitro* culture. However, PPNP also has a unique characteristic for removing sheets of epithelial cells from culture vessels. This lead to the increased use of this enzyme to improve understanding of the basic biology of skin and as an enzyme to prepare cells or biomaterials for therapeutic use.

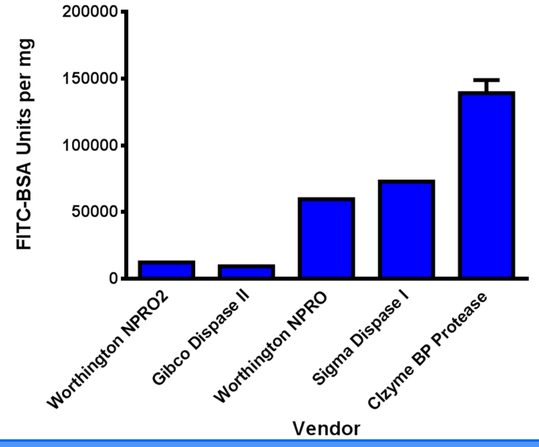
Although PPNP has been used for over 30 years for these therapeutic applications, knowledge about the biochemistry of this enzyme is scattered in different reports. During the development of a purified PPNP enzyme, VitaCyte isolated a different form of this enzyme that has a 1.9 to 2.3 fold higher specific activity than the current purified commercial enzymes. This poster summarizes the characterization of this new form of PPNP and the basic biochemical characteristics and biological applications of these enzymes.

Reagents and Methods

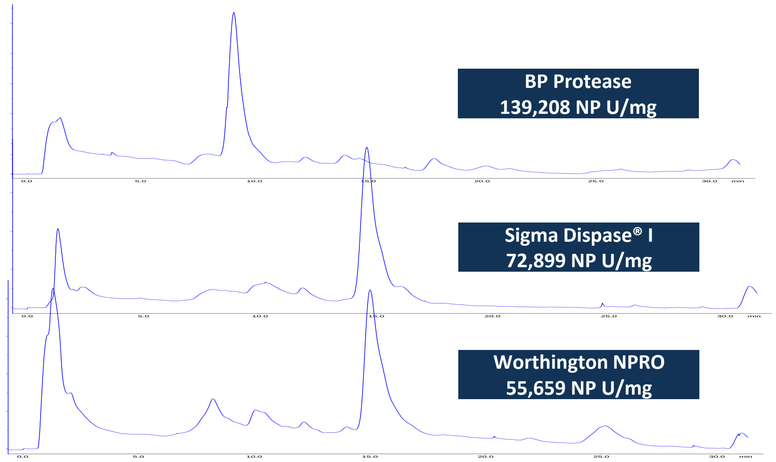
PPNP was isolated from *P. polymyxa* culture supernatants using a culture media that contained no animal derived materials. The purified PPNP was prepared using proprietary methods. The enzyme activity was determined by using a fluorescent microplate assay using fluorescein isothiocyanate covalently bound to bovine serum albumin (FITC-BSA) as substrate. PPNP containing samples were diluted into a fixed dose of substrate, incubated at 35°C, and fluorescent readings taken at 30 second intervals. As the substrate is degraded, there is an increase in FITC fluorescence as measured by fluorescent units (FU). The average of multiple kinetic reading (FU/min) were used to calculate an enzyme activity. One unit = increase of 1 FU per minute. The specific activity was determined by calculating the FU per mg where 1 mg = 1 A_{280}/mL. VitaCyte purified PPNP (BP Protease) was compared to other purified PPNP enzymes using the enzyme analysis described above and by using a modified analytical anion exchange chromatography procedure initially described by Hefley.

Results

Crude PPNP was obtained from Gibco (Dispase II) or Worthington Biochemicals (NPRO2). Purified PPNP was obtained from Sigma (Dispase I) or Worthington Biochemicals (NPRO). A comparison of the specific activities of these enzymes is shown below. As expected, the crude enzymes have a specific activity about 10% of the specific activities found with the purified NPRO and Dispase I. By contrast, the BP Protease has a specific activity 1.9 and 2.3 fold higher than the NPRO and Dispase I, respectively.



One explanation for the results reported on the left is the different purities of the enzymes. This is an acceptable explanation for the differences in the crude and purified forms of Dispase and NPRO enzymes. However, further analysis of those purified enzymes with BP Protease showed that a different molecular forms of PPNP detected by analytical anion exchange chromatography likely accounted for this difference.



Biological applications

Application	Description includes desired outcome	Human Cells	Additional Enzyme(s)
Cell isolation PPNP alone	Recovery of cells from tissue	Epidermal skin cells Limbal epithelial cells Hepatocytes Islets Adipose	None None Collagenase Collagenase Collagenase
Sub-cultivation	Recovery of adherent cells from tissue culture vessels, used for maintenance or expansion of cultured cells	Mammalian cell lines Newborn skin epithelial Adenoma derived epithelial sheets	None None None
Cell disaggregation	Dissociation of cells from isolated cell structures (e.g., islets)	Islets Adrenal medulla (chromaffin)	None None
Disease models	Posterior vitreous detachment Chemotherapy	N/A	None None
Biomaterials	Removal of cells from membranes	Amniotic membrane	None

Enzyme Selectivity

Studies performed using human amniotic membranes treated with or without PPNP for 30, 60, or 120 min at 37°C, then stained for specific proteins using immunofluorescent methods showed that at 30 min, PPNP alone degraded type IV & VII collagen, thrombospondin, elastin, fibronectin, tenascin, and laminin. By contrast, collagen types I & II were not degraded and type VI collagen was degraded after 120 min of digestion.

Conclusions

- Higher activity preparation of PPNP enzyme prepared, difference likely due to minimal degradation of PPNP
- Cost per unit activity of BP Protease is 50 and 500 fold lower than the NPRO and Dispase I, respectively, selling it at a price per U competitive with crude PPNP