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The Role and Efficiency of Ammonium Sulphate Precipitation in Purification Process of Papain Crude Extract

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Abstract

It has been common to do fractionation (for example using ammonium sulphate as a precipitating agent) before doing a more sophisticated method for purification of a protein. The logic behind this is easy to understand, but in fact, the precipitation step often causes severe loss in yield and activity of the protein, making the whole purification effort too costly. In this work we evaluated the specific activity (thus, purification factor) and total activity (yield) during the purification process of papain from a crude extract using ion exchange chromatography (IEC), with and without prior fractionation using ammonium sulphate. Detail assays in each step were recorded and SDS-PAGE was also done to reveal the protein profile of the purification products.

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Keywords: Ammonium sulphate; Precipitation; Purification

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Nomenclature

μmol	micromol (mol x 10^{-6})
μg	microgram (gram x 10^{-6})
min	minute
ml	millilitre (litre x 10^{-3})

1. Introduction

The latex of *Carica papaya* is a rich source of the cysteine endopeptidases, including papain, glycylendopeptidase, chymopapain and caricain, which constitute more than 80% of the whole enzyme fraction[1]. Papain (EC 3.4.22.2) is a minor constituent (5–8%) among the papaya endopeptidases[1–3]. The enzyme is used widely as a meat tenderizer, and has also several other applications, e.g. for defibrinating wounds, treatment of edemas, shrink proofing of wool, etc. Purification of papain from papaya latex has traditionally been achieved by precipitation methods[4–6], however, the purified enzyme still remains contaminated with other proteases. An alternative purification strategy has involved the use of various chromatographic techniques including ion exchange, covalent, or affinity chromatography[1,7–10].

Initial observation showed that the fractionation process using ammonium sulphate actually frequently causes a quite significant loss of papain. In this work, we analyzed the role and the utility of doing fractionation using ammonium sulphate before subsequent ion exchange chromatography, in terms of specific activity (thus, purification factor) and total activity (yield).

2. Methods

2.1 Materials

The papaya fruit, *C. papaya* was grown locally in Surabaya, Indonesia and used as starting latex material. Polyacrylamide, bis-acrylamide, casein and ammonium sulphate were purchased from Sigma–Aldrich (St. Louis, USA), while other reagents were bought from Merck.

2.2. Isolation of latex from C. papaya

Fresh latex was collected from papaya fruit. Initially, four tosix longitudinal incisions were made on the unripe fruit using a stainless steel knife. The exuded latex was allowed to run down the fruit and drip into collecting devices attached around the trunk. Following collection, the latex was transferred to a plastic bottle and stored at −20°C.

2.3. Ammonium sulphate precipitation

The latex extract was centrifuged for 15 minutes at 2500 x *g*, at room temperature. The supernatant obtained was centrifuged at 5000 x *g* for 15 minutes at room temperature to obtain clean liquids. Proteins were sequentially precipitated from 50 mL of this crude extract by stepwise addition of solid ammonium sulphate with stirring at a certain degree of saturation, followed by incubation on ice for at least 2 h and centrifugation at 10,000 x *g* at 4°C for 15 minutes. The pellet obtained after each centrifugation was resuspended in max. 10 mL of buffer containing 10 mM phosphate, pH 7. Those steps above were done for 50, 60, 70 and 80% of ammonium sulphate saturation, respectively. Aliquots of precipitated fractions were analyzed for its protein concentration, enzyme activity and MW by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). As comparison, part of the crude extract was also directly subjected to the same analysis.

2.4. Determination of protein concentration

The protein content in the samples during purification was determined by the Bradford method[11].