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In vitro Activity of Supercoiled Double Stranded DNA Cleavage by Proteins Extracted from Sweet Potato (*Ipomea Batatas* L.) Peel

Retno Arianingrum

Department of Chemistry Education, Faculty of Mathematic and Natural Science, Yogyakarta State University, Karangmalang Yogyakarta, 55281 Indonesia
*Corresponding author.E-mail: Arianingrum@lycos. com., Tel: +62-0274-484638

ABSTRACT

This research was aimed to identify the presence of RIPs (Ribosome Inactivating Proteins) in sweet potato (*Ipomea batatas* L.) through its activity test to cleave the supercoiled double stranded DNA. The leaf, tuber and peel of white, yellow, and purple sweet potatoes were extracted using 5 mM sodium phospate buffer (pH 7) containing 0.14 M sodium chloride then were precipitated using amonium sulfate and were purified by dialysis. Each extract was measured its protein contents with Lowry method using BSA solution as standard. The activity of supercoiled DNA cleaved was determined using pUC 19 plasmid DNA. The activity test was done using agarose gel electrophoresis by observing 3 criterions viz. the decreasing thinkness band of supercoiled DNA, the band of circular form thickening and the appearance of the linear band which were subsequently compared to the plasmid DNA without treatment. The extract of *Mirabilis jalapa* leaf was used as a positive control. The result showed that only peel of white, yellow, and purple sweet potato had RIPs activity in cleaving the supercoil DNA. Increasing concentrations of the crude extract resulted in increasing the activity, which was indicated by the decreasing thinkness band of supercoiled DNA, and the thickening of circular and linear band.

Key words: Ribosome-Inactivating Proteins (RIPs), sweet potato (*Ipomea batatas* L.), and supercoil DNA

INTRODUCTION

Sweet potato (*Ipomea batatas* L.) is one of plants that has many varieties and useful as food by society in Indonesia. The tuber of this plant has many colour such as white, yellow, and purple depend of their varieties. Sweet potato is the source of carbohydrate, especially in the form of starches (22.4%) and sugar (24%). In this plant also contain protein (1.43%), fat (0.17%), food fiber (1.6%), calcium (29 mg/100g), phosphorus (51 mg/100 g), iron (0.49 mg/100 g), vitamin A (0.01 mg/100 g, vitamin B1 (0.09 mg/100 g, vitamin V (24 mg/100 g), and water (83.3 g). Based on several research, in sweet potato contain secondary metabolites that have many biological activities, such as phenolic as anti cancer, anti mutagenic (Yashimoto *et al.*, 2002), anti diabetes (Kusano *et al.*, 2001), and anti radical

(Yashimoto *et al.*, 2004). The plan also uses as traditional medicine especially red and purple sweet potato to treat jaundice, swelling, and xeropthalmia.

Ribosome-inactivating protein (RIPs) is a group of toxic proteins produced in plants. RIPs, as indicated by name, inactivate eukaryotic ribosomes by cleaving the N-glycosidic bond at the A_{4324} position of the 28S RNA fraction so that they are no longer function in protein synthesis. RIPs can be classified into two major types according to their structure; type 1 consists of a single chain with molecular weight of around 30 kDa, while type 2, with a molecular weight of around 60 kDa, usually consists of two chains (A and B) connected by a disulfide bond. The A chain is homologous to type 1 RIP and is responsible for the toxicity of the molecule. The B chain is a lectin which binds to the cell surface and facilitates the entry of A chain into the cell (Barbieri *et al.*, 1993). In the plants, RIPs distributed in seed, tuber, peel, leaf, stem, and sap (Stirpe and Battelli, 2006).

Beside the N-glycosidase activity, RIPs are also capable to cleave supercoiled double stranded DNA become nicked circular and linear form. RIPs only act on supercoiled and nicked-circular, and seldom cleave the linear DNA (Ling *et al.*, 1994). The cleavage activity on supercoiled double stranded DNA was first reported with trichosantin, an abortifacient, immunosuppressive and anti tumor protein purified from the traditional Chinese herb medicine Tian Hua Fen (Li *et al.*, 1991). Interest in RIPs is growing due to several discoveries, such as the antiviral activity of mirabilis antiviral protein (MAP), a type 1 RIP which has a potent cytotoxicity, which make them excellent activities for cancer therapy (Goldmacher *et al.*, 1994; Tang *et al.*, 2003). Several researches also indicated that RIPs is more toxic in cancer cell than normal cell (Pneumans *et al.*, 2001). These many activities in RIPs emerge expectation to find a new selective anticancer drug.

Sweet potatoes are already known to have many biological activities. So far, the research about their protein activities has not yet been much investigated. The aim of the research is to identify the presence of RIPs (Ribosome Inactivating Proteins) in sweet potato (*Ipomea Batatas* L.) through the cleavage of supercoiled double stranded DNA testing.

METHODOLOGY

Material

Ipomea batatas L. plants were obtained from Kendal area; Ngawi, East Java, and *Mirabilis jalapa* leaf were collected from a Yogyakarta State University garden. Plasmid pUC 19 were obtained from laboratory stock of Parasitology Laboratory Gadjah Mada University. Plasmid pUC 19 were isolated from *Escherichia coli* (*E.coli*) DH5α that contain pUC resulted from transformation process by Sambrook method (1989). The bacteria were cultured in LB medium containing this ampicilin 150 μg/ml at 37°C. After reaching the stationary growth phase, total plasmid DNA was purified by modified alkaline lysis procedure.

Preparation of *Ipomea batatas* L protein extract from leaf, tuber and peel

Protein extract of leaf, tuber and peel from white, yellow, and purple *Ipomea batatas* L. were prepared by grinding in 0.14 M sodium chloride in 5 mM sodium phospate buffer pH 7.2. The extract was strained and centrifuged (10,000 rpm, 10 menit). The supernatant (crude extract) was separated from the sediment, followed by protein precipitation using ammonium sulphate at 100% saturation. The precipitated proteins were then dissolved in sodium phospate buffer pH 7.2 and dialysed to removed the salt. Each extract was measured for its protein contents with Lowry method using BSA (Bovine Serum Albumin) as a standard solution.

Cleavage of supercoiled DNA by the protein extract

Three µl plasmid DNA (pUC 19) and 1 µl buffer TMN (50 mM Tris-HCl; 10 mM $MgCl_2$; 100 mM NaCl), pH 8.0 were incubated with various amount of extract/RIPs at room temperature for 1 hour. At the end of reaction, 3 µl loading buffer (30% glycerol; 200 mM EDTA; 0.25% bromophenol blue; and 0.25 xylene cyanol FF) were added. Electrophoresis was carried out in 0.5 x TBE buffer in a 0.8% agarose gel. DNA bands were visualized by staining with ethidium bromide. *Mirabilis jalapa* extract was used as positive control.

RESULTS AND DISCUSSION

The leaf, tuber and peel of white, yellow, and purple sweet potato were used in this study. The protein content of each sample was determined by Lowry method before testing for the presence of RIPs in this sample. The identification was done by observing their ability to cleave supercoiled DNA (pUC 19) using agarose gel electrophoresis based on 3 criterions viz. the decreasing thinkness band of supercoiled DNA, the circular band thickening and the appearance of the linear band which were subsequently compared to the plasmid DNA without treatment. The extract of *Mirabilis jalapa* leaf was used as a positive control.

1.1 Protein extract

The protein content in the leaf, tuber and peel of white, yellow, and purple sweet potato was represented in Table 1. It was indicated that the highest protein contain was in tuber yellow sweet potato.

Table 1 Protein content of the leaf, tuber, and peel of white, yellow, and purple sweet potato

Sweet Potato	Protein content (mg/ml)		
	Leaf	Tuber	Peel
White	4.22	13.87	6.40
Yellow	3.05	**20.56**	11.93
Purple	4.64	13.82	7.96

1.2 Cleavage of supercoiled DNA by the protein extract

The results in Figure 1 indicated that double stranded supercoiled DNA was cleaved by the protein extract of sweet potato peel. The cleavage of supercoil DNA activity seemed to be concentration dependent, as shown by the decrease of supercoil form followed by the increase of nicked circular one. At low concentrations of the protein extract, the supercoiled DNA band in the agarose gel became gradually fainted, while nicked bands began to appear (Figure 1-3). The supercoiled DNA completely disappeared at the concentration of 0.5 mg/ml (Figure 1 lane 7), 0.6 mg/ml of (Figure 2 lane 6), and 0.4 mg/ml (Figure 3 lane 5). The result suggests that sweet potato peel contains RIPs protein.

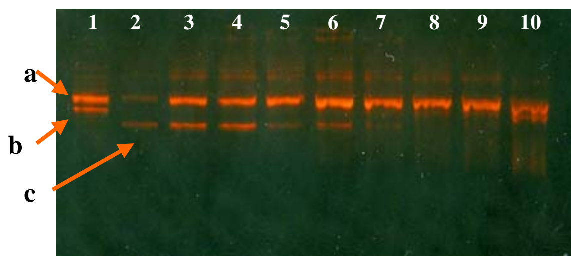

Figure 1 The electrophoregram of cleaving of supercoiled pUC 19 by peel protein extract of white sweet potato. Lane 1 treated pUC 19 with *M. jalapa* (positive control), lane 2 Untreated pUC 19, line 3 to 10 treated with sweet potato protein extracts at a concentration of : 0.03, 0.06, 0.2, 0.3, 0.5, 0.6, 1, and 2 mg/ml respectively.
Note : (a) linear DNA; (b) nicked circular DNA, and (c) supercoiled DNA

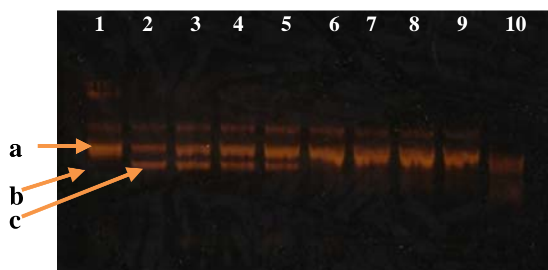

Figure 2 The electrophoregram of cleaving of supercoiled pUC 19 by peel protein extract of yellow sweet potato. Lane 1 treated pUC 19 with *M. jalapa* (positive control), lane 2 untreated pUC 19, lane 3 to 10 treated with sweet potato protein extracts at a concentration of: 0.05, 0.1, 0.3, 0.6, 0.8, 1.0, 2.0, and 4 mg/ml respectively.
Note : (a) linear DNA; (b) nicked circular DNA, and (c) supercoiled DNA

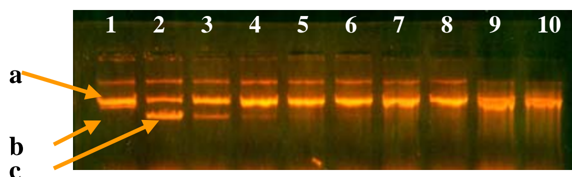

Figure 3 The electrophoregram of cleaving of supercoiled pUC 19 by peel protein extract of purple sweet potato. Lane 1 treated pUC 19 with *M. jalapa* (positive control), lane 2 Untreated pUC 19, lane 3 to 10 treated with sweet potato protein extracts at a concentration of : 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1, and 2 mg/ml respectively.
Note : (a) linear DNA; (b) nicked circular DNA, and (c) supercoiled DNA

On the other hand, no activity was found when pUC 19 was treated with the protein extract isolated from leaf of white, yellow, and purple sweet potatoes, even when the concentrations of protein extracts were increased up to 1.2 mg/ml for white, and 1 mg/ml for yellow and purple sweet potatoes. Also there was no activity when pUC 19 treated with protein extract of tuber of white, yellow, and purple sweet potatoes, even when the concentrations of protein extracts were increased up to 10 mg/ml for yellow, and 7 mg/ml for white and purple sweet potatoes. It was indicated that protein extracts isolated from leaf and tuber of sweet potato do not contain RIPs protein.

The cleavage of supercoiled DNA is characteristic of both type 1 and type 2 RIPs, in addition to N-glycosidase activity (Li *et al.*, 1991; Ling *et al.,* 1994). These results suggested that sweet potato peel contains RIPs protein. Several lines of evidence suggest that the anti-tumor, anti-viral, and anti-parasitic effect of plant proteins, such as gelonin or pokeweed antiviral protein (PAP) which are RIPs, are not solely due to the N-glycosidase activity, which is remove an invariant adenine in a conserved loop in the 28 S rRNA (Peumens *et al.*, 2001). There is an alternative effect of RIPs which posses a single-stranded adenine DNA glycosylase activity (Barbieri *et al.*, 1997). It is possible that the protein fractions containing RIPs activity from sweet potato peel might be responsible for the anticancer activity. While there is still no direct evidence that this activity contributes to cytotoxicity, the ability of RIPs to damage DNA by removal of normal, non-mispaired bases in vitro distinguished them from the other members of DNA glycosylase family, which might also contribute to the activity (Nicholas *et al.*, 1998).

It was demonstrated that RIPs were able to cleave supercoiled double stranded DNA at the same site of rRNA (Wang and Tumer, 1999). This evidence was supported by He and Liu (2004) which demonstrated that mutants of recombinant cinnamomin A-chain devoid of N-terminal 52 or/and C-terminal 51 amino acid residues lost both the activity of RNA N-glycosidase and the ability to release adenines from supercoiled DNA, as well as the ability to cleave supercoiled DNA into nick and linear forms. It suggested that phospodiester bonds in the extensively deadenylated region of supercoiled DNA would become fragile and

liable to be broken spontaneously owing to the tension in supercoiled DNA (Barbieri *et al.*, 2000; He and Liu, 2004). However to prove this, further studies have to be carried out to observe its N-glycosidase activity and inhibition of protein synthesis.

CONCLUSION

The result indicated that in the leaf, tuber and peel of white, yellow, and purple sweet potato *(Ipomea batatas* L.); only peel contained RIPs proteins, which was indicated by their ability to cause supercoiled double stranded DNA cleavage. Increasing concentrations of the crude extract resulted in increasing the activity, which was indicated by the decreasing thinkness band of supercoiled DNA, and the thickening of circular and linear band.

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