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Short Communication

Depolymerisation of mucilage isolated from *ruredzo* (*Dicerocaryum zanguebarium*) by ascorbic acid in the presence of catalysts

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Abstract

No change was observed in the viscosity of samples of mucilage from *Dicerocaryum zanguebarium* when treated with 5 mM ascorbic acid. Mucilage was treated with ascorbic acid in the presence of copper and iron, and a mixture of the two ions. The effect of treatment was followed by gel filtration of products on a Sepharose 6B column and by measuring the amount of reducing sugars generated by the dinitrosalicylic acid (DNSA) method. The molecular weight of treated samples was lower than that of untreated controls. The amount of reducing groups was found to increase. When the mucilage was treated in a system containing hydrogen peroxide and copper ions, the polymer was also degraded, yielding fragments of lower molecular weight as reflected by the broadened gel filtration peak.

1. Introduction

For quite some time, we have been interested in the potential applications of the polysaccharide that is isolated from the leaves of *ruredzo* (*Dicerocaryum zanguebarium*). *Ruredzo* is a creeping plant that grows widely in sandy soils of Southern Africa and is traditionally used as food and medicine (Benhura and Marume, 1993). This plant appears to be a suitable candidate for the commercial exploitation of its mucilage, after the mucilage has been properly characterised. We believe that most of the uses of *ruredzo* depend on the presence of copious amounts of mucilage that occur in the leaves of the plant.

The mucilage from *ruredzo* is a pectic polymer with a molecular weight of about 500 000 dalton (Benhura and Marume, 1993). The polysaccharide contains galactose and xylose in approximately equal amounts, in addition to arabinose and a small proportion of mannose. The protein and uronic acid content is 2.1% and 8.1% respectively.

It was reported that ascorbic acid-transition metal ion systems generate oxygen radicals that are involved in the depolymerisation of polysaccharides. It is suggested that, in the presence of transition metal ions, ascorbic acid takes part in the formation of the ·OH radical that is involved in the depolymerisation reaction (Uchida and Kawakishi, 1986). In this study, we investigated the effect of ascorbic acid in the presence of transition metal ions on solutions of the mucilage from *ruredzo*.

2. Experimental

2.1. Preparation of mucilage

The mucilage was extracted from dried *ruredzo* leaves with boiling water, and purified using copper acetate, as described previously (Benhura and Marume, 1993). The dry mucilage was obtained either by freeze-drying solutions of the purified polymer or by precipitating the polysaccharide with ethanol and drying in an oven at 100°C. Dry mucilage was ground and kept at room temperature until required.

2.2. Effect of ascorbic acid on viscosity of mucilage

A stock solution of mucilage 1% (w/v) was prepared by dissolving 1 g of mucilage in 100 ml of distilled water. The mucilage was left overnight to hydrate completely in a refrigerator at 4°C. The final concentration of the solution of mucilage was determined by drying in an oven at 100°C. Ascorbic acid (AsA) was added to a 0.5% (w/v) solution of mucilage to a achieve concentration of up to 5 mM.

2.3. Effect of ascorbic acid on viscosity of mucilage in the presence of copper and iron ions

Ascorbic acid (0.33 mM) was added to a 0.5% (w/v) solution of mucilage in the presence of iron sulphate (3.3 μM), copper sulphate (3.3 μM), and a mixture of the two salts at

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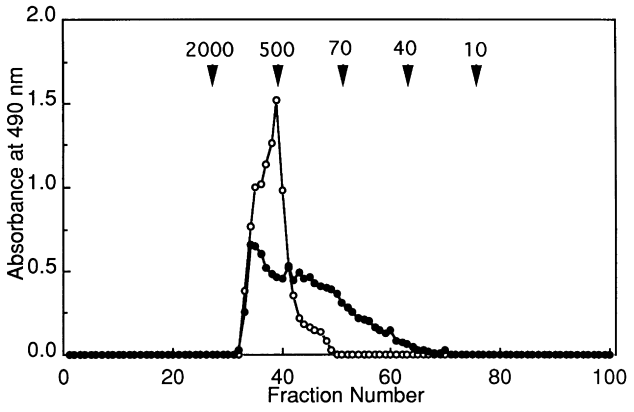

Fig. 1. Gel filtration of 0.05% (w/v) mucilage before (–○–) and after (–●–) treatment with 2 mM AsA and 10 μM copper ion in 0.07 M phosphate buffer, pH 7.2 on a 40 cm × 3 cm Sepharose 6B column that was eluted with 1 M NaCl at a flow rate of 30 ml/h at room temperature. The arrows show the positions of the dextran standards.

the same concentration. The control was a solution of mucilage that contained only ascorbic acid. The reaction mixtures were incubated at 30°C for up to 5 h (Matsumura and Pigman, 1965). The viscosity was measured at room temperature using an Ostwald type viscometer. The relative viscosity was calculated from the efflux times of the samples and distilled water.

2.4. Preparation of the reaction systems

The reaction in the AsA-copper ion system was carried out in 50 ml of 0.07 M phosphate buffer, at pH 7.2, containing 0.05% mucilage, 2 mM AsA, and 10 μM $CuSO_4$ and the reactions in the H_2O_2-copper ion system contained 0.05% mucilage, 1 mM H_2O_2, and 10 μM $CuSO_4$. The reaction mixtures were kept at room temperature for about 24 h, and the reaction was stopped by adding EDTA (40 μM). The reaction mixtures were dialysed overnight against 5 l of distilled water. The non-dialysable part was freeze-dried in a Christ Alpha 2–4 model freeze drier and reconstituted to make 1% solutions (Uchida and Kawakishi, 1986).

2.5. Colorimetric measurement of the depolymerisation of mucilage

The course of depolymerisation of mucilage induced by the AsA-copper ion and the H_2O_2-copper ion systems was followed by measuring the amount of reducing groups that were generated using the dinitrosalicylic acid (DNSA) method (Chaplin, 1986). Aliquots (1.5 ml) were removed during incubation of the reaction mixtures at room temperature for about 24 h. To samples, standards and controls (100 μl), 1 ml of DNSA reagent was added, and the mixture heated for 10 min in a boiling water bath. The mixture was cooled rapidly to room temperature under running tap water. The absorbance was measured at 570 nm in a Shimadzu UV 160 A spectrophotometer (Chaplin, 1986).

2.6. Gel filtration

The column (40 cm × 3 cm) was packed with Sepharose 6B and the homogeneity of the bed tested by running Blue Dextran (2 mg/ml, 500 μl). The quality of packing was checked by observing the progress of a zone of blue dextran through the bed (Pharmacia handbook). The void volume of the gel was calculated using either the peak recorded by the UV chart recorder or by finding the total volume of the fractions collected when the Blue dextran was eluted. T dextran standards from Pharmacia (1% w/v, 500 μl) were used as standards. Fractions (3 ml) were collected at a flow rate of 30 ml/h. The native mucilage (1% w/v, 500 μl) and mucilage treated with either AsA-copper ions or H_2O_2-copper systems (1% w/v, 500 μl) was applied onto the column and eluted with 1 M NaCl (Uchida and Kawakishi, 1986).

2.7. Determination of carbohydrate

The concentration of the eluted carbohydrates was determined colorimetrically by the phenol–sulphuric acid method (Chaplin, 1986). To samples, standards and controls (600 μl) were added, 5% phenol (600 μl) and concentrated sulphuric acid (3 ml). The reaction mixtures were allowed to stand for 10 min, vortexed and were then incubated for 30 min at room temperature. The absorbance of the samples was read at 490 nm in a Shimadzu UV 160 A spectrophotometer.

3. Results and discussion

When AsA was added by itself, in the presence of copper, iron or a mixture of the two ions, to a 0.5% (w/v) solution of mucilage of 2 mM concentration, there was no appreciable change in viscosity. The relative viscosities of polysaccharides usually decrease when organic acids are added. The decrease in viscosity of the polysaccharide is related to the pH change (Isobe et al., 1992).

When copper or iron ions were added in the presence of AsA, the rate of depolymerisation of hyaluronic acid increased (Matsumura and Pigman, 1965). Matsumura and Pigman also reported that by adding only AsA to the hyaluronic acid, the fluidity-time curve of the polymer was found to be similar to that of the polymer only.

When the native mucilage was run through the column, its peak occurred at a position corresponding to a molecular weight of 500 000 dalton. The molecular weight of mucilage that was treated with AsA-copper ion system (the reaction stopped immediately with EDTA), was more or less the same as the native mucilage. For the reaction mixture which was incubated for 24 h at room temperature, the peak was found to be broader, indicating that fragments of lower

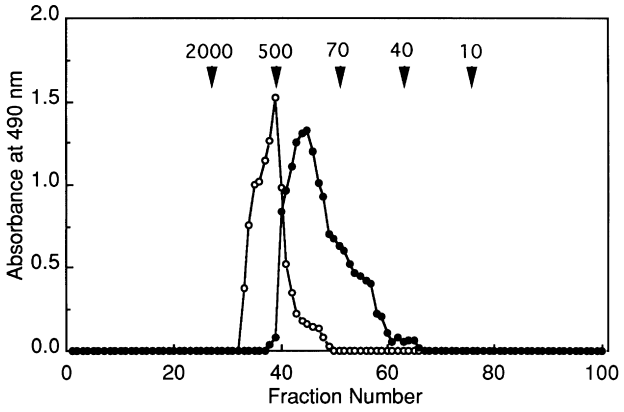

Fig. 2. Gel filtration of 0.05% mucilage before (–○–) and after (–●–) treatment with 1 mM H_2O_2-10 μM copper ion system in 0.07 M phosphate buffer, pH 7.2 on a 40 cm × 3 cm Sepharose 6B eluted with 1 M NaCl at a flow rate of 30 ml/h.

molecular weight were generated as a result of the action of the AsA-copper ion system. The effect of the AsA-copper ion system on *ruredzo* mucilage is shown in Fig. 1.

The molecular weight of the mucilage that was treated with H_2O_2-copper ion system shifted to lower values (Fig. 2). Peripheral residues were depolymerised leaving the main chain intact. Such a mechanism of action would explain why little change was observed in the viscosity of treated samples.

The depolymerisation of mucilage by AsA-copper ion systems were comparable to that reported by Uchida and Kawakishi (1986) for dextran, hyaluronate, pectin, and pullulan. The depolymerisation of the mucilage was confirmed by the colorimetric measurement of changes of reducing power (Fig. 3). The result showed a large increase in reducing power due to the degradation of the polymer. The mucilage treated with AsA-copper ion system had more reducing groups produced than the mucilage treated with H_2O_2-copper ion system. The difference could be caused by the position of degradation, whether it was main chain and branches, or the branches only.

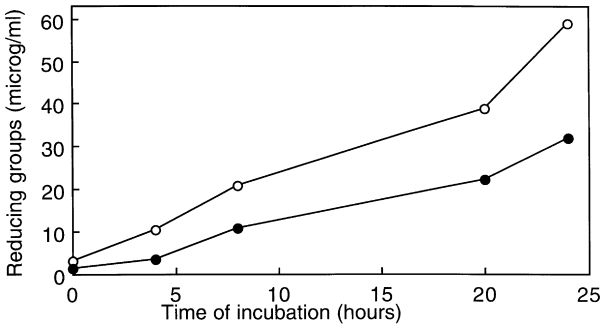

Fig. 3. Reducing groups of 2 mM AsA, 10 mM copper ion (○) and 1 mM copper ion (●) systems were determined by the dinitrosalicylic acid method and expressed in terms of equivalent of galactose.

Acknowledgements

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