



Characterization of the Polysaccharide from Adhesive Discs of *Parthenocissus tricuspidata*

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A water-soluble polysaccharide (PT2) was isolated from adhesive discs of *Parthenocissus tricuspidata* and its structure was determined by monosaccharide analysis, methylation, periodate oxidation, Smith degradation and IR spectrum. Some advanced structure of PT2, such as honeycomb structure and individual polysaccharide, were observed by atomic force microscopy (AFM). We also evaluate the adhesive property of PT2 and the results suggested that it was a potential bio-adhesive with the highest adhesion force value of 295.8 nN.

Key Words: *Parthenocissus tricuspidata*, Adhesive disc, Polysaccharide, AFM.

INTRODUCTION

Parthenocissus tricuspidata belongs to the family of Vitaceae, is a tendril-climber liana known for its adhesive disc. Because of its remarkable climbing ability, *P. tricuspidata* is mainly used as garden plant to green and beautify the environment. In fact, *P. tricuspidata* is a potential resource of mucopolysaccharides¹, which is secreted from adhesive discs and enables the whole plant to strongly affix to different surfaces, e.g., rock, tree and wall. The researches concerning the adhesive property of *P. tricuspidata* have been widely carried out since Darwin's time¹⁻⁸. However, the details of the mucopolysaccharide from discs are still unknown. As a result, the adhesion mechanism of *P. tricuspidata* can not be determined thoroughly. Herein, a water-soluble polysaccharide (PT2) from adhesive discs of *P. tricuspidata* was identified by a series of chemical and physical methods. Especially, we employed AFM to further characterize the PT2 and obtained some advanced structures under different concentrations. In order to reveal the relationship between the attachment strength and the polysaccharide, the adhesion force was also measured by AFM in contact mode. This findings will be helpful to reveal the nature of the mucopolysaccharide secreted from adhesive discs and the biological attachment systems of *P. tricuspidata*. More important, we expect to develop a bionic adhesive material which can "walk" along different supports with high adhesive ability like *P. tricuspidata*.

The adhesive discs of *P. tricuspidata* were collected from Guangzhou, Guangdong Province of mainland China. After pretreating with ethanol to remove lipids and pigments, the dried residual sample was extracted with hot water (80 °C, 3 h)

for three times and then 95 % ethanol was added gradually to precipitate three fractions named PT1, PT2 and PT3, respectively. The polysaccharides were deproteinized by Sevag method⁹. Due to the highest yield, PT2 was selected for further purification on a SephadexG-100 column, eluted with 0.1 mol/L NaCl at a flow rate of 0.5 mL/min. PT2 showed a single homogeneous peak and the average molecule weight was estimated to be 204 kDa by GPC.

The results of monosaccharide analysis for PT2 were shown in Table-1. Compared to the retention time of the references, PT2 was mainly composed of fructose, galactose and arabinose in a molar ratio of 4:3:2. The IR spectrum of PT2 showed main absorption bands for hydroxyl groups (3420 cm⁻¹), C-H stretch (2941 cm⁻¹), the glycosidic linkage ν(C-O-H) and ν(C-O-C) contributions (1145, 1074, 1025 cm⁻¹), β- and α-type glycosidic linkages (891 and 838 cm⁻¹)¹⁰⁻¹³.

TABLE-1
MONOSACCHARIDE ANALYSIS OF POLYSACCHARIDE

Components	Retention time (min)	
	Reference material	PT2
Rhmnose	10.589	
Arabinose	11.355	11.244
Xylose	12.031	
Fructose ^a	15.530, 16.120	15.493, 15.976
Galactose	15.907	15.819

^aFructose can generate two alditol acetates derivatives (mannitol acetate and glucitol acetate).

Periodate oxidation was carried out according to the method of Dixon and Lipkin¹⁴. The consumption of NaIO₄ (1.18 mol) was much more than the production of HCOOH

(0.25 mol), indicating that 1,2 or 1,4-glycosidic linkages existed in PT2 besides the 1,6-glycosidic linkage. After Smith degradation, the analysis of GC gave glycerol, indicating that the C-4 hydroxy group of the glycosyl residues in PT2 was free. Therefore, PT2 mainly contained 1,2- and 1,6-glycosidic linkages. In addition, galactose was also found by GC, which was attributed to (1 → 3)-linked galactosyl residues.

In order to further confirm the linkage of polysaccharide, PT2 was methylated according to the method of Ciucanu and Kerek¹⁵ and analyzed by GC-MS. On the basis of the database and previous reports¹⁶⁻¹⁸, methylation analysis of PT2 showed 1,3,4,6-Me₄-Man, 1,3,4,6-Me₄-Glu, 3,4,6-Me₃-Man, 3,4,6-Me₃-Glu, 2,4,6-Me₃-Gal, 2,4-Me₂-Gal, 2,3,5-Me₃-Ara, 2,3-Me₂-Ara corresponding to (2 →)-linked and (2 → 1)-linked Fru residues, (1 → 3)-linked and (1,3 → 6)-linked Gal residues as well as non-reducing terminal and (1 → 5)-linked Ara residues.

In this paper, we especially employed AFM to determine the adhesive property and the advanced structure of PT2. AFM experiments were performed on a Nanoscope Multi-SPM IIIa (Digital Instruments, Santa Barbara, USA) at ambient conditions. Drops of polysaccharide solution (2 μL) were deposited onto freshly cleaved mica surfaces and dried in air for one day before experiments. A thin film was formed on mica surface when the concentration of PT2 was 1 mg/mL (Fig. 1). Forty force-distance curves of PT2 were collected in contact mode and the data of adhesion force ranged from 248.4-295.8 nN and an average value was 254.3 nN by calculation with spring constant (0.58 N/m). In order to ensure that the adhesion force was dominated by the polysaccharide, we also measured the adhesion force on a bare mica surface, which was only about 38 nN. Therefore, the adhesion force of PT2 was mainly originated from the polysaccharide. We found that the adhesive force of PT2 was nearly close to the adhesive strength of geckel adhesive¹⁹, indicating that PT2 was a potential resource of a bio-adhesive.

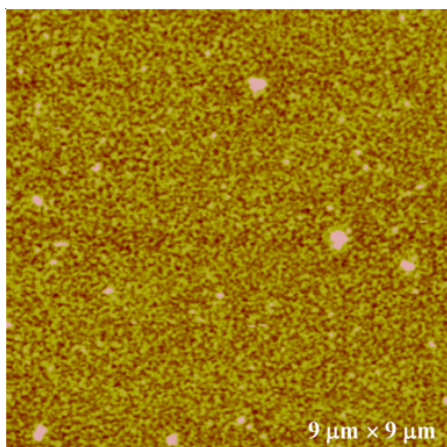


Fig. 1. AFM image of the thin film formed by PT2 under the concentration of 1 mg/mL

The advanced structures of PT2 under different concentrations were observed by AFM in tapping mode. As shown in Fig. 2a, PT2 formed the network that resembled a honeycomb structure at the concentration of 0.1 mg/mL, which was different from those formed by κ-carrageenan, xanthan or gellan in shape²⁰. As the polysaccharide solution was diluted to 0.01

mg/mL, a type of heterogeneous structure, which consisted of large aggregates connected by tenuous branched strands, appeared on the mica surface (Fig. 2b). This destroyed network confirmed that the reduction of polysaccharide concentration would inhibit the formation of network²¹. In addition, we also found that the individual polysaccharide chains could self-assemble into random twining structure and the average height of stand was about 0.480 nm (Fig. 3).

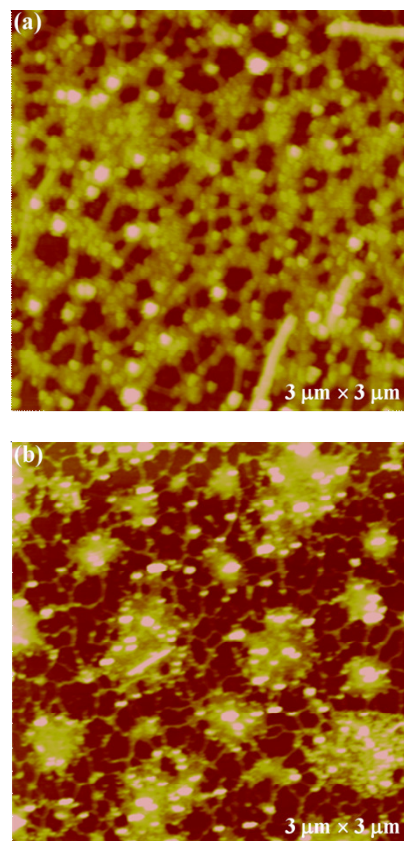


Fig. 2. AFM images of polysaccharide network under the concentration of (a) 0.1 mg/mL and (b) 0.01 mg/mL

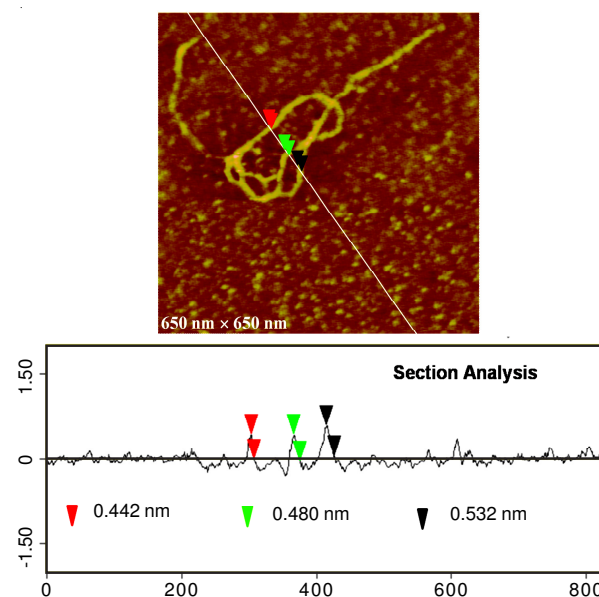


Fig. 3. AFM image of random twining structure formed by individual polysaccharide

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REFERENCES

1. A.G. Endress and W.W. Thomson, *Can. J. Bot.*, **55**, 918 (1977).
2. C. Darwin, *The Movements and Habits of Climbing Plants*, John Murray, London (1875).
3. A.G. Endress and W.W. Thomson, *Protoplasma*, **88**, 315 (1976).
4. S. Junker, *New Phytol.*, **77**, 741 (1976).
5. A.J. Bowling and K.C. Vaughn, *Protoplasma*, **232**, 153 (2008).
6. T. Steinbrecher, E. Danninger, D. Harder, T. Speck, O. Kraft and R. Schwaiger, *Acta Biomater.*, **6**, 1497 (2010).
7. T.X. He, W.W. Yang and W.L. Deng, *Prog. Nat. Sci.*, **18**, 1220 (2008) in Chinese.
8. T.X. He, L. Zhang and W.L. Deng, *Arch. Biol. Sci.*, **63**, 393 (2011).
9. M.G. Sevag, D.B. Lackman and J. Smolens, *J. Biol. Chem.*, **124**, 425 (1938).
10. F.S. Park, *Application of IR Spectroscopy in Biochemistry, Biology and Medicine*, Plenum Press, New York, p. 100 (1971).
11. M.B. Wu, Y.L. Wu, J. Zhou and Y.J. Pan, *Food Chem.*, **113**, 1020 (2009).
12. X.H. Chen, Y.H. Liu, X. Bai, L. Wen, J.B. Fang, M. Ye and J.C. Chen, *J. Nat. Prod.*, **72**, 1988 (2009).
13. S.A. Baker, E.J. Bourne, M. Stacey and D.H. Whiffen, *J. Chem. Soc.*, 171 (1954).
14. J.S. Dixon and D. Lipkin, *Anal. Chem.*, **26**, 1092 (1954).
15. I. Ciucanu and F. Kerek, *Carbohydr. Res.*, **131**, 209 (1984).
16. G.L. Sasaki, P.A.J. Gorin, L.M. Souza, P.A. Czelusniak and M. Iacomini, *Carbohydr. Res.*, **340**, 731 (2005).
17. X.M. Wu, H. Dai, L.X. Huang, X.M. Gao, K.W.K. Tsing and P.F. Tu, *J. Nat. Prod.*, **69**, 1257 (2006).
18. X.H. Chen, Y.H. Liu, X. Bai, L. Wen, J.B. Fang, M. Ye and J.C. Chen, *J. Nat. Prod.*, **72**, 1988 (2009).
19. H. Lee, B.P. Lee and P.B. Messersmith, *Nature*, **448**, 338 (2007).
20. A.R. Kirby, A.P. Gunning and V.J. Morris, *Biopolymers*, **38**, 355 (1996).
21. V.J. Morris, A.P. Gunning, A.R. Kirby, A. Round, K. Waldron and A. Ng, *Int. J. Biol. Macromol.*, **21**, 61 (1997).