Arch. Biol. Sci., Belgrade, 63 (2), 393-398, 2011 DOI:10.2298/ABS1102393H

BIOLOGICAL ADHESION OF *PARTHENOCISSUS TRICUSPIDATA*

TIANXIAN HE, LI ZHANG and WENLI DENG

College of Materials, Science and Engineering, South China University of Technology, Guangzhou 510640, PR China

Abstract - *Parthenocissus tricuspidata* is a climbing plant of the grape family. It can climb with its adhesive discs on different substrates such as stone mountains, roadside stone banks, exterior walls of buildings, thereby withstanding strong winds and storms without detachment. The details about the adhesion process of *Parthenocissus tricuspidata* are not yet entirely understood. We studied the component-structure-property relationship of the adhesive discs in detail and propose a two-stage model to describe the biological adhesion: (i) structural contact and (ii) adhesive action. These two stages and their variations play an important role for the attaching of the adhesive disc to different structural surfaces. We believe that in *Parthenocissus tricuspidata* different mechanisms work together to allow the adhesive disc to climb on various vertical substrates and reveal strong adhesive properties.

Key words: *Parthenocissus tricuspidata*, adhesive disc, biological adhesion, component-structure-property.

UDC 582.6/.9:581.5

INTRODUCTION

There are many examples of smart biological adhesion systems that are effectively adapted to their unique function and allow for permanent or temporary adhesion under different environmental conditions. During the last decade, research was mainly focused on reversible adhesion in animals, e.g. insects (Watson et al., 2005), spiders (Zheng et al., 2010), tree frogs (Voyles et al., 2009), geckos (Autumn et al., 2000) and mussels (Lee et al., 2007). In many land species, the functionality of the reliable attachment systems is dependent on contact-splitting by developing tiny hair-like structures with a thickness of several nanometers to form van der Waals forces between these structures and the substrate (Autumn et al., 2000). Marine mussels rely on the excretion of various chemical substances to obtain adhesion (Lee et al., 2007). Plants and especially climbing plants, which also have adhesion systems with excellent mechanical properties, are rarely studied as to the component-structure-property relationship of their adhesive organs (Zhang et al., 2008; He et al., 2010). This holds especially for detailed bioinformatics analyses of the biological adhesion of permanent attachment found in climbing plants.

Parthenocissus tricuspidata (*P. tricuspidata*) is a climbing plant belonged to the grape family and grows on stone mountains, roadside stone banks and the exterior walls of buildings. The amazing sticking ability of *P. tricuspidata* has attracted great interest for centuries. As early as 320 years ago, Malpighi first accurately described the tendril of *P. tricuspidata* in the chapter 'de capreolis et consimilibus vinculis' from his 'opera omnia'. His record was later extended macroscopically by Darwin and Ewart and microscopically by Mohl, Lengerken and others (Junker, 1976). Since Darwin's work (Darwin, 1875), surprisingly few studies have dealt with the adhesion mechanism of *P. tricuspidata* (Endress and Thomson, 1977; Bowling and Vaughn, 2008). As we know, the tendrils of *P. tricuspidata* become swollen at their tips and flatten against the substrate upon contact, concomitant with a secretion of an adhesive fluid. Earlier findings confirmed that this adhesive fluid is most likely composed of an acidic mucopolysaccharide (Endress and Thomson, 1977). In a recent report, modern immunocytochemical methods have shown that the individual components of the adhesive substance is mainly composed of debranched rhamnogalacturonan / that accumulate at the point of contact between the substrate and the epidermal cells (Bowling and Vaughn, 2008).

According to Darwin's record, a 10-year-old branchlet with only one remaining adhesive disc attached to a wall could support a weight of 2 lb without the disc detaching (Darwin, 1875). Its distinctiveness continues to inspire our enthusiasm to further investigate the chemical composition of the adhesive disc and the mechanism of adhesion, as well as its potential applications in novel adhesive and bionics science. Herein, our study focuses on a detailed analysis of the componential and functional aspects of the adhesive disc and the climbing mechanism of *P. tricuspidata*, and presents a model to explain how *P. tricuspidata* is able to climb on various vertical surfaces up to a height of 20 m.

MATERIALS AND METHODS

Plant material

The plant material used in this study was grown indoors in the greenhouses of the botanical garden of South China Agricultural University, Guangzhou city, Guangdong province, China. 148 fresh adhesive discs were grown on and collected from 20 pieces of silicon wafer (5 × 5 cm) covered by a sealed plastic film as the basic climbing substrates.

Organic components analysis

After the adhesive discs were pulled down from the silicon wafers, the residual layer on the substrates was extracted with methanol, and successively with ethyl acetate three times at room temperature, respectively. The extracts were combined and evaporated under reduced pressure at 50C, yielding a crude sample which was then subjected to silica gel chromatographic column (200 g, 4 cm diameter) eluted with light petroleum-ethyl acetate mixtures (10:1 to 1:10) to yield 2 fractions in accordance with the gradient. These two fractions were analyzed by High-Performance Liquid Chromatography/Mass Spectrometry (Esquire HCT PLUS, Bruker, Germany).

Metal elements analysis

The adhesive discs were pretreated with ultrasonic waves and washed to clean before use for analysis. The sample (700 mg) was immersed in 60 ml aqua regia in a 100 ml beaker for 48 h. Then it was heated up to dryness on an electric platen, and the beaker was washed with 1% HNO_3 and deionized water. After filtration, it was titrated to 6 ml and subjected to Atomic Absorption Spectrometer (Z-5000, Hitachi, Japan).

RESULTS AND DISCUSSION

Organic components

Cytochemical and modern immunocytochemical methods have been used to study the histological structure of the adhesive disc of *P. tricuspidata*. Endress and Thomson suggested that the adhesive substance secreted by the epidermal cell of the adhesive disc is most likely composed of an acidic mucopolysaccharide; insoluble carbohydrates also occur in the adhesive disc, but with little or no protein or lipid in it (Endress and Thomson, 1977). Afterward, Bowling and his co-workers revealed that the adhesive may be produced from the selective modification and remobilization of the wall components of the adhesive disc cells, primarily a debranched rhamnogalacturonan I, and a limited synthesis of new components so as to form a complex and effective adhesive (Bowling and Vaughn, 2008). But to date, to the best of our knowledge, there are no chemical analysis reports published on the adhesive disc of *P. tricuspidata*. We first used high-performance liquid chromatography/mass spectrometry (HPLC/MS) to preliminarily analyze the chemical composition of the adhesive residues on the silicon wafer substrates.

HPLC/MS has been applied widely in various fields such as natural product and drug analysis. Minor or trace constituents, which are difficult to obtain by conventional phytochemical methods, could be detected by mass spectrometry (Zhang *et al.*, 2008). Fig. 1 shows a total ion chromatogram of the adhesive residue extraction obtained by HPLC/MS. Clearly, there are at least 21 components in the adhesive residues. Because the residual layer on the silicon wafers is quite small, a comprehensive composition analysis is very difficult. Therefore, preliminary empirical formulas of the 21 compounds were considered to replace it. These compounds could have empirical formulas assigned using MS and MS/MS data in the extract. The chemical formulas of composition are listed in Table 1. The empirical formulas calculated are based on the observed assumed M + H ion in the MS data and the MS/MS fragment ion data. For an empirical formula to be accepted as being likely to be correct, the MS/MS spectra must include fragments ions that are consistent with the proposed empirical formula from the MS data. This is a higher criterion to pass than if only MS data were required.

The formulas demonstrate that most of the 21 compounds contain nitrogen, sulfur and oxygen. These compounds are widely known for their ability to generate polarity. It is likely that the typical hydrophobic, saturated hydrocarbon 'tail' with a polar 'head' molecular structure is expressed. Because most of the substrates that *P. tricuspidata* normally or typically climb on are stone mountains, roadside stone banks, exterior walls of buildings, etc., which are inorganic or at least polar in nature, the composition of adhesive residues suggests that the adhesive disc may have a weak adhesion of hydrogen bonding to attach to different substrates.

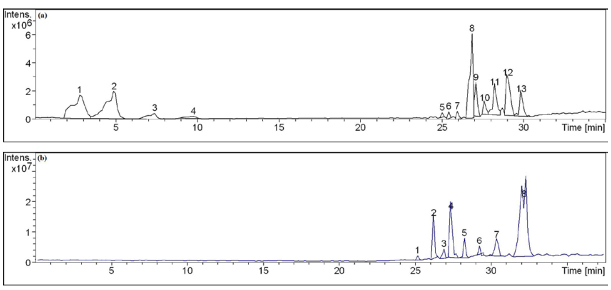

Fig. 1. A total ion chromatogram. (a) fraction 1, (b) fraction 2.

Table 1. Empirical formulas for the 21 components in the adhesive residues as determined by HPLC/MS/MS analysis

fraction 1	compound formula	fraction 1	compound formula
1	$C_{33}H_{29}N_2$	1	$C_{14}H_{30}N_2O_3$
2	$C_{31}H_{53}N_2$	2	$C_{20}H_{10}N_2O_3$
3	$C_{29}H_{21}N_6$	3	$C_{10}H_{18}N_{12}O_3$
4	$C_{27}H_{17}N_8$	4	$C_{14}H_{21}N_8$
5	$C_{29}H_{22}O_{11}$	5	$C_{16}H_{25}N_8$
6	$C_{31}H_{40}O_7$	6	$C_{23}H_{15}NO_3$
7	$C_{37}H_{39}N_4$	7	$C_{21}H_{21}N_{10}$
8	$C_{25}H_{11}N$	8	$C_{25}H_{31}N_4O_2$
9	$C_{16}H_{29}N_5O_3$		
10	$C_{26}H_{29}NO_7$		
11	$C_{19}H_{14}N_5O_2$		
12	$C_{26}H_{32}N_2O_8$		
13	$C_{16}H_{17}N_3S$		

Metal elements

The metal element metabolism of plants has been extensively studied and the basic information on many topics is available in monographs on plant physiology or plant nutrition. The metabolic fate and role of each metal element in plants can be characterized in relation to some basic processes such as uptake and transport within a plant, enzymatic processes, con-

Table 2. The content of 6 metal elements found in the adhesive disc of *P. tricuspidata*

metal element	K	Na	Ca	Mn	Fe	Mg
content (μg/g)	2562.86	463.71	7397.14	25.03	774.00	508.29

centrations and forms of occurrence, deficiency and toxicity, ion competition and interaction (Kabata and Pendias, 1992).

During the growth and development of *P. tricuspidata*, metal elements are required in smaller quantity and based on the established biochemical functions. Six metal elements were identified in the adhesive disc of *P. tricuspidata* (Table 2). In general, K is a macronutrient; Na can regulate the stomata switch; Mg is a component of the chlorophyll in all green plants; it is essential for photosynthesis and it may also help activate many plant enzymes needed for growth; Fe is a key catalyst in chlorophyll production and Mn participates in nitrogen metabolism (Kabata and Pendias, 1992). However, a perplexing question is why Ca is present in larger amounts (almost three times than K) than any other metal element,

In addition to acting as an essential part of plant cell wall structure, Ca may also have an influence on the surface adhesion of *P. tricuspidata*. The central role of Ca^{2+} in biological processes has been well established (Simkiss, 1974). The function of Ca^{2+} depends on its ability to bind to other molecules and change their properties. The important properties of Ca^{2+} are its concentration, orbital structure, binding strength with a particular ligand and the rate constant involved in the binding. It can be argued that the sum product of these properties governs the role of Ca^{2+} in any particular situation.

Metal binding exists in biological adhesion systems. There is evidence that Ca^{2+} and other divalent cations regulate adhesion in a wide range of bacteria. Ca^{2+} also regulates adhesion and motility in diatoms, and this is consistent with its role as a universal regulator of cellular function in all prokaryotes and eukaryotes studied to date (Geesey et al., 2000). For instance, adhesion of encysting zoospores of *Phytophthora cinnamomi* was enhanced by increasing the Ca^{2+} concentration from 2 to 20 mmol/l (Gubler et al., 1989). As a matter of fact, Ca^{2+} can bind with rhamnogalacturonan I to form a complex that has high affinity (Scanlan et al., 2010). Based on the report of Bowling and Vaughn who discovered that rhamnogalacturonan I is the main component of adhesive fluid secreted by the epidermal cells of adhesive discs of *P. tricuspidata* (Bowling and Vaughn, 2008), it can be inferred that Ca^{2+} ions seem to be involved in non-specific interactions such as the neutralization of the electrical double layer between the epidermal cell and the substrate surface as well as specific adhesive interactions that cannot be replaced by other cations. The unique properties of Ca^{2+} ions promote both specific and non-specific interactions with protein and polysaccharide adhesive molecules at the interface.

Adhesion model

Each tendril of *P. tricuspidata* is made up of a main axis with five to nine branchlets alternately attached; at the tip of the branchlets there is a small swelling, which after the stimulus of contact develops into an adhesive disc (Fig. 2a). The super adhesive effect of the adhesive disc is attributed to the joint action of its composition and structure. On one hand, the adhesive disc has contact-splitting structures; on the other hand, it can secrete mucilage from the epidermal cells. Based on the componential and structural data of our study, we propose a two-stage model for the adhesion mechanism of *P. tricuspidata*.

The first stage of adhesion is the initial contact formation of the elongated epidermal cells — clusters of finger-like structures (Fig. 2b) — of the adhesive disc with the climbing substrate. These elongat-

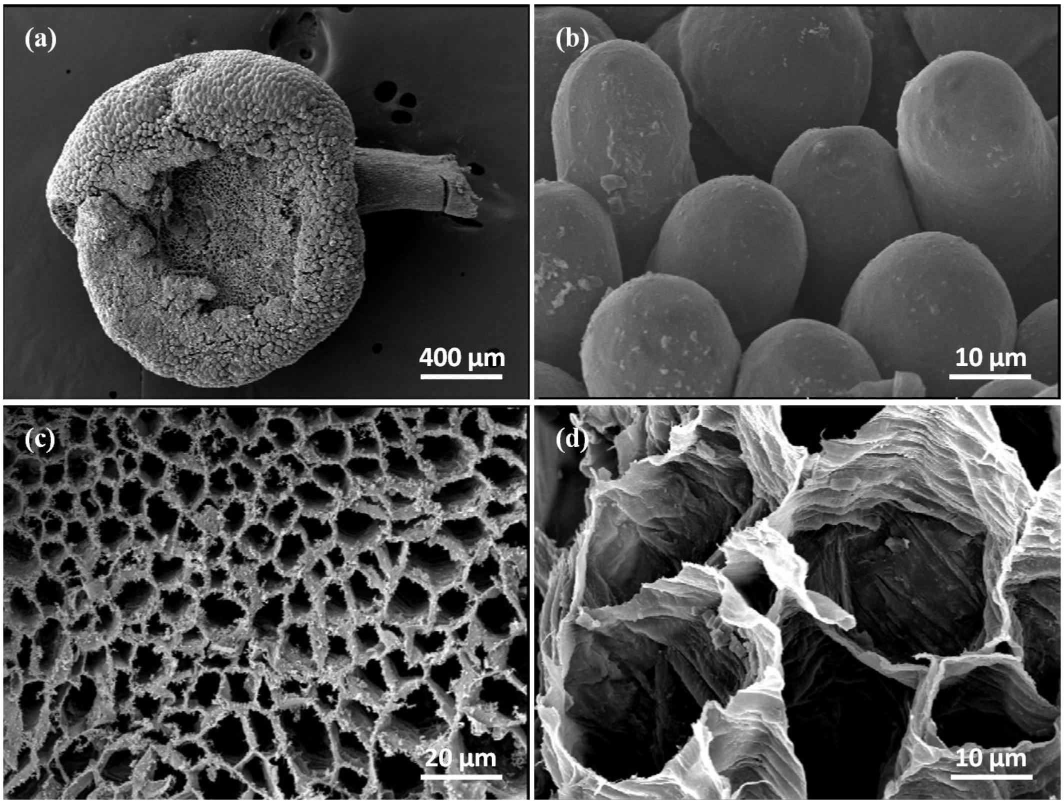

Fig. 2. Scanning electron microscopy images. (a) adhesive disc, (b) clusters of finger-like structures, (c and d) microchannels inside the adhesive disc.

ed epidermal cells are analogous to the crampon of a climber's boot; they force themselves into the rugged surface and mold the pad to the shape of the support, and in this way they have a friction in the vertical direction. It is not possible that these structural changes of the epidermal cells of the adhesive disc can generate a large enough adhesive force, but it has a localized and assistant effect for the final permanent adhesion. However, we do not know yet what the exact 'signal tunneling' contact to the substrate is and how it may be mediated to initiate the second stage of adhesion.

The initial contact triggers the secretion of adhesive fluid from the epidermal cells of adhesive disc. The microchannels inside the adhesive disc (Fig. 2c, d) take charge of the accumulation and transportation of mucilage, which results in the adhesive fluid occupying the space between the substrate and the cells as well as the intracellular spaces between the epidermal cells. It enlarges the contact area between the adhesive disc and the substrate. Therefore, the extensive quantities as well as general distribution of adhesive fluid along the walls and in the intracellular spaces around all the epidermal cells of adhesive disc improve the extreme resistance, thus avoiding the adhesive disc from easily detaching from substrates by pulling and shear forces.

Lee's group has reported a hybrid biologically inspired adhesive consisting of an array of nanofabricated polymer pillars coated with a thin layer of a synthetic polymer that mimics the adhesive proteins found in mussel holdfasts (Lee et al., 2007). The adhesion of the nanostructured polymer pillar arrays increased over 3-fold when coated with the mussel-mimetic polymer. This composite adhesive, which contains the salient elements of gecko structure and mussel adhesives, is very similar to the attachment system of *P. tricuspidata* and could be a helpful support for our proposed adhesion model.

There may be some mechanisms that cannot be currently demonstrated. However, we believe that, in *P. tricuspidata*, different mechanisms work together to allow the adhesive disc to attach to various substrates and reveal super adhesive properties. Understanding the biological adhesion of *P. tricuspidata* has great scientific significance, it may open up new research and application in biomechanics, novel adhesive and bionics science and technology.

CONCLUSIONS

In summary, 21 organic components and 6 metal elements were experimentally explored in the adhesive layer and disc of *P. tricuspidata*. Considering that most of the 21 compounds contain nitrogen, sulfur and oxygen, hydrogen bonding would be a weak adhesion for *P. tricuspidata* climbing. In the 6 metal elements, the calcium content is the most and calcium ions may be involved in the process of adhesion. We studied the component-structure-property relationship of the adhesive disc of *P. tricuspidata* in detail and propose a two-stage model to describe the biological adhesion: (i) structural contact and (ii) adhesive action. These two stages and their variations work together and play an important role for the adhesive disc attaching to different structural surfaces. Understanding the super-adhesion mechanism of *P. tricuspidata* is a prerequisite for bio-inspired design of adhesive materials, and more experimental and theoretical works are imperative to fully open this new research field.

Acknowledgments - This work was supported financially by the National Natural Science Foundation of China (No. 51073059 and No. 91023002), State Key Development Program for Basic Research of China (No. 2009CB930604).

REFERENCES

Autumn, K., Liang, Y. A., Hsieh, S. T., Zesch, W., Chan, W. P., Kenny, T. W., Fearing, R. and *R. J. Full* (2000). *Nature* **405**, 681–685.

Bowling, A. J, and *K. C. Vaughn* (2008). *Protoplasma* **232**, 153-163.

Darwin, C. (1875). *The movement and habits of climbing plants*, John Murray, London.

Endress, A. G. and *W. W. Thomson* (1977). *Can J Bot* **55**, 918-924.

Geesey, G. G., Wigglesworth-Cooksey, B. and *K. E. Cooksey* (2000). *Biofouling* **15**, 195-205.

Gubler, F., Hardham, A. R, and *J. Duniec* (1989). *Protoplasma* **149**, 24–30.

He, T. X., Zhang, L., Xin, H. L. and *W. L. Deng* (2010). *Pure Appl Chem* **82**, 91-96.

Junker, S. (1976). *New Phytol* **77**, 741-746.

Kabata-Pendias, A, and *H. Pendias* (1992). *Trace elements in soils and plants*, CRC Press, Boca Raton.

Lee, H., Lee, B. P. and *P. B. Messersmith* (2007). *Nature* **448**, 338-344.

Scanlan, E. M., Mackeen, M. M., Wormald, M. R, and *B. G. J. Davis* (2010). *J Am Chem Soc* **132**, 7238-7239.

Simkiss, K. (1974). *Endeavor* **33**, 119-123.

Voyles, J., Young, S., Berger, L., Campbell, C., Voyles, W. F., Dinudom, A., Cook, D., Webb, R., Alford, R. A., Skerratt, L. F. and *R. Speare* (2009). *Science* **326**, 582-585.

Watson, F. L., Puttmann-Holgado, R., Thomas, F., Lamar, D. L., Hughes, M., Kondo, M., Rebel, V. I. and *D. Schmucker* (2005). *Science* **309**, 1874-1878.

Zhang, M. J., Liu, M. Z., Prest, H. and *S. Fischer* (2008). *Nano Lett* **8**, 1277-1280.

Zheng, Y. M., Bai, H., Huang, Z. B., Tian, X. L., Nie, F. Q., Zhao, Y., Zhai, J, and *L. Jiang* (2010). *Nature* **463**, 640-643.