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Mussel adhesive protein as an environmentally-friendly harmless wood furniture adhesive

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ABSTRACT

Recent adhesive technologies have focused on the development of high-quality and eco-friendly adhesives. Thus, there is a gradual shift from the currently used chemical-based adhesives toward harmless adhesives with improved quality and performance. Here, we evaluated the potential use of bacteria-produced recombinant mussel adhesive protein (MAP) as a harmless wood furniture adhesive. We formulated a MAP wood adhesive as an inclusion body type for economical preparation, and we confirmed its harmlessness through the non-detection of volatile organic compounds and heavy metals. The formulated MAP showed sufficiently strong bulk adhesive strength for the dried gluing of wood adherends. We also found that the formulated MAP wood adhesive exhibits robust adhesion in various environmental conditions, including open assembly times, incubation times, temperatures, and humidity levels. In summary, the developed recombinant MAP could be successfully used as a promising environmentally-friendly, harmless wood furniture adhesive.

1. Introduction

Chemical-based adhesives have been widely used in most household and industrial fields due to their outstanding adhesive strengths and low production costs [1]. However, they generate volatile organic compounds (VOCs) and contain harmful components such as formaldehyde, phenol, and trichloroethane, which are regarded as endocrine-disrupting chemicals that might cause health hazards such as sick house syndrome and atopic dermatitis [2–7]. Although many efforts have been made to reduce harmful components, such as VOCs and endocrine-disrupting chemicals, chemical-based adhesives have a limitation in terms of toxicity. Thus, current chemical-based harmful adhesives are continuously shifting toward environmentally-friendly adhesives [8,9]. Biological adhesives derived from nature, such as tannin, casein, soybean, lignin, gelatin, and fibrin, have been suggested as alternative adhesives [10–15]. Differently from chemical-based adhesives, biological adhesives are distinctly regarded as environmentally-friendly and non-toxic adhesives, but limitations relating to relatively low adhesive properties and high production costs remain [16–18].

Mussel adhesive proteins (MAPs) have attracted interest for their various potential uses as adhesives in cell and tissue engineering, biomedicine, and biotechnology [19–23]. These adhesive biomaterials have been regarded as environmentally friendly due to their non-toxic, biocompatible, and biodegradable properties. However, their applications have been greatly limited due to the difficulty of obtaining sufficient quantities. Previously, a genetically redesigned recombinant hybrid MAP was produced in considerable quantities using a bacterial expression system with significant adhesion ability [24] and was successfully applied for diverse purposes [25–33]. In the present work, we evaluated the potential application of a recombinant MAP as an environmentally-friendly adhesive for wood-based furniture through investigations of its harmlessness and adhesion properties under various conditions.

2. Materials and methods

2.1. Preparation of inclusion body and powder MAPs

Escherichia coli BL21 (DE3) cells expressing recombinant MAP were cultured in 150 L Luria-Bertani (LB) medium with 50 μg/mL

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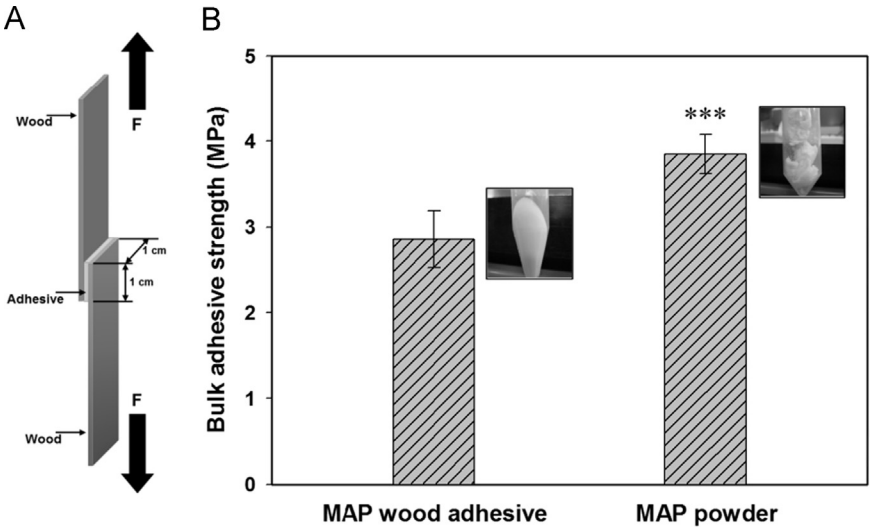

Fig. 1. (A) Schematic illustration of the measurement of bulk shear strength with wood adherends and (B) bulk adhesive strengths of MAP wood adhesive and MAP powder. Adhesive strengths were measured after 24 h of curing of attached wood adherends at room temperature and 60% relative humidity without an open assembly time. Data are shown as means and standard deviations (N=minimum of 8).

ampicillin (Sigma-Aldrich, St. Louis, MO, USA) in a 300 L fermentor (Fermentec, Cheongju, Korea) at 37 °C and 250 rpm. When a cell density (OD_{600}) reached approximately 0.2–0.5, 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG; Sigma-Aldrich) was added to induce the expression of recombinant MAP. After 9 h of incubation, the cells were harvested by centrifugation at 18,000*g* for 10 min at 4 °C. The harvested cell pellet was resuspended in 5 mL lysis buffer (10 mM Tris–Cl and 100 mM sodium phosphate; pH 8.0) per gram (wet weight) and lysed by a cell disruption system (Constant Systems, Daventry, UK) at 20 kpsi. Cell debris was centrifuged at 18,000*g* for 20 min at 4 °C to collect the inclusion body. The inclusion body MAP was stored in −80 °C before further analyses. To obtain powder MAP, the inclusion body was washed with TTE buffer (1% TritonX-100, 1 mM EDTA, 0.1 mM PMSF, and 50 mM Tris–HCl; pH 8.0) followed by distilled water (DW) and resuspended in 25% (v/v) acetic acid. The acetate-extracted soluble MAP was dialyzed twice in DW and freeze-dried to obtain powder. Powder MAP was also stored in −80 °C before further analyses. Production yields for inclusion body and powder MAPs were determined as ~10.5 g/L (~64% purity) and ~0.4 g/L (~93% purity), respectively.

2.2. Measurement of bulk adhesive strength

Bulk adhesive strength was measured using wood adherends (5 mm thickness × 10 mm width × 150 mm length) according to a previously described protocol [25,34]. Briefly, the inclusion body and lyophilized powder MAPs were dissolved in DW for a final concentration of 300 g/L. The samples were applied onto 10 × 10 mm^2 areas of wood surfaces (Fig. 1A) as a single spread method, and the attached wood adherends were directly incubated with compressed clip for 24 h at room temperature (25 ± 3 °C) for sufficient curing. In addition, to test practical wood adhesion, bulk adhesive strength was measured in various conditions, such as incubation time, open assembly time, temperature, and humidity, after 3 days of incubation. A commercially available chloroprene (CR) adhesive (Ogong Bond, Incheon, Korea) was used as a comparative chemical-based wood adhesive. Shear strength was directly measured using a universal material testing machine (Model No. 3344; Instron, Norwood, MA, USA) with a 2000 N load cell (Fig. 1A). Adhesion force in Pascal (Pa) was calculated through dividing the shear force (in Newton) by the adherend overlap area (in m^2) following the ASTM D1002 standard method (ASTM International D1002-05, 2005). Each adhesion measurement was repeated at least 10 times and averaged for a given sample. The statistical significance was analyzed by comparing two groups through the paired Student's t-test: statistical significance is designated by $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.005$. The certified determination of the bulk adhesive strength for the inclusion body MAP was conducted at the Korea testing laboratory (KTL, Seoul, Korea), an officially authorized institution, following a protocol almost the same as that described in this work, except the attached wood adherends were stored for 2 weeks at room temperature before testing. Shear strength was measured using a universal material testing machine (Model No. 5589; Instron) at 23 ± 1 °C and 60% relative humidity.

2.3. Harmlessness evaluation of MAP wood furniture adhesive

To measure the possible emission of harmful contaminants, 1.2 g of inclusion body MAP was coated on a glass slide with a 63 × 63 mm^2 area, washed with DW, and incubated for 3 h at 100 °C. Then, the extracted sample was taken from the slide and placed in a 20 L chamber at 25 °C and 50% relative humidity with 0.5 times/h air change. VOCs and formaldehyde were analyzed by gas chromatography/mass spectrometry (GC/MS; Shimadzu, Kyoto, Japan) and high-performance liquid chromatography (HPLC; Agilent, Santa Clara, CA, USA), respectively. A harmlessness evaluation was conducted at KTL. In addition, the heavy metal contents (lead (Pb), arsenic (As), cadmium (Cd), mercury (Hg), and chromium (Cr)) from the inclusion body MAP was analyzed by inductively coupled plasma-mass spectrometry (ICP-MS; Agilent) after sample pretreatment using a microwave digestion system.

3. Results and discussion

3.1. Selection of formulation type for wood furniture adhesive

For successful use as a practical wood furniture adhesive, the selection of a feasible formulation of MAP is a prerequisite. In the present work, we compared two formulation types (inclusion body and powder) of a MAP wood furniture adhesive that are derived from MAP production processes. Because the recombinant MAP was expressed as an inclusion body in *E. coli* [20], we evaluated an inclusion body MAP as a candidate for a potential