Loss of phosphate determines the versatility of spider orb-web glue ball

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Abstract

Spiders capture their prey by weaving an “invisible” orb-web which has both adhesive and fixed properties. Different types of silk in the orb-web have different functions, wherein the key to capturing a prey is the ball-like glue (glue ball), which coats the silk strands. This glue ball has highly versatile properties, but the mechanisms leading to its versatility remain unclear. The salts found in the web have been previously suggested to play an important role in terms of viscosity, not water. However, the distribution of salt and water in the glue ball has not yet been directly observed. Here, we mapped the salts in different states using a homemade Time-of-Flight Secondary Ion Mass Spectrometer (TOF-SIMS) with a high lateral resolution. To our surprise, the glue ball was found to contain little water. The functional transformation of the glue ball from a viscous glycoprotein (capturing prey) to a hardened protein (retaining prey) relies solely on the stimulation of mechanical forces. The phosphate is a key factor for its versatility.

Keywords: Spider silk, intelligent materials, mechanochemistry, biomolecular engineering, TOF-SIMS, FIB.
Introduction

After hundreds of millions of years of evolution, spiders have developed ingenious structures both on the macro- and micro-scale. Among the more than 40,000 species of spider, the most common is the orb-weaver spider in our daily life. This spider contains a variety of secretory glands that secrete different types of silk for various uses, with different functions and structures associated with each secretory gland. The spiders first weave radial lines with a high-strength to create the skeleton of their web. Subsequently, they start creating their web by weaving in spiral lines with a good tensile strength, which can absorb the powerful kinetic energy of the flying prey. The spiral lines were coated with a glue used for capturing prey. This glue is initially evenly coated on the spiral lines; however, the glue on the spiral lines forms equal-spaced ball-like structures via self-organization (Fig S1). The appearance of glue balls can be traced back to 130 million years ago, to the early Cretaceous period. Despite this, our understanding of its nature is still very superficial. The glue ball is a versatile structure, wherein the spiral line stretches quickly when hit by flying prey. The glue droplets become very viscous after coming into contact with the prey, thereby providing adhesion. Then, the movement of the prey as it struggles to break free from the web helps to transforms the glue into an elastic solid material, effectively retaining the prey. The mechanisms and structural models leading to the formation of the glue ball remain unclear. According to the recent reports, the main components of the glue ball are glycoproteins and salt. Two potential structural mechanisms have been previously suggested. The first suggests that concentrated glycoprotein is wrapped in a salt and water shell. The argument behind this mechanism is that the salt adjusts the water content of the glue ball, thereby maintaining and absorbing atmospheric water to promote protein binding with water. The second mechanism suggests that salt and protein are present throughout the glue ball, such that vapor pressure due to high salt...
concentrations is at levels close to those of the ambient humidity of the spider’s habitat. The salt then prevents interface failure under high humidity conditions and isolates atmospheric water. This moisture resistance maintains a constant elasticity and adhesive strength even when the humidity changes. It appears that the distribution of salt and water is key to determining the multifunctional mechanism of the glue ball. The methods currently used for its analysis are mainly based on optical methods and nuclear magnetic resonance (NMR), which do not allow for the direct observation of the distribution of salt and water in the glue ball. The glue ball on the silk strands and the glue ball attached to the substrate are very different in terms of structure, as determined using optical microscopy. This means if the sample is processed before measurement, even if it is simply attached to the substrate, its properties will change. Previous attempts to understand the properties of these adhesives have neglected the fact that the critical structural deformation of the glue ball itself causes a change in its properties. We directly observed the distribution of salt and water in glue balls at different states using a homemade Time-of-Flight Secondary Ion Mass Spectrometer (TOF-SIMS) with a high lateral resolution. Furthermore, we identified the mechanism for fixing the prey after coming into contact with glue balls by analyzing the salt distribution property.

**Experimental**

**Samples**

Sample collection was carried out during the night from July 2018 to September 2018, on the road along the Kawaguchi River (coordinates (WGS84): E139.31, N35.68), Hachioji, Tokyo. Samples were obtained from the orb-web of the spider *Araneus ventricosus* (Fig. 1). In order to obtain a wide area of the web, we picked up the orb web using a large copper ring. We then cut off the silk from outer circumference of the ring, taking care not to pull or deform the silk in
the retaining ring (Fig. 1). Finally, we placed spiral line silks on a piece of aluminum with a
diameter of 10 mm and stored it in a liquid nitrogen storage tank (for rapid freezing) or a
vacuum chamber (for vacuum drying).

TOF-SIMS and cross-sectioning with FIB
See reference \textsuperscript{26} for a detailed description.

Rapid freezing
We used circulating liquid nitrogen to cool the stage to -120°C. We placed the sample holder
and holder cap together in liquid nitrogen, which were cooled thoroughly, and closed the cap in
liquid nitrogen. The sample holder was then placed in a load-lock chamber and evacuated to
10^{-4} \text{ Pa}. Nitrogen gas was excluded by high pressure due to the evaporation of the liquid
nitrogen from the cap. The sample was not contaminated by outside air. The cap was opened in
the load-lock chamber under 10^{-4} \text{ Pa}. Finally, the sample was pushed to the stage in main
chamber (10^{-6} \text{ Pa}). (Fig. S2)

Nanoparticles firing at the glue balls
We placed palladium nanoparticles in a high pressure air bucket and aimed at the orb-web from
a distance of 10 cm. The nanoparticles were ejected through the tip of the mouth and directed at
the orb-web. Any spiral lines hit by the nanoparticles were obtained and placed on an aluminum
substrate. (Fig. S3)

Results and Discussion

For our study, we used a common spider typically found in cities, \textit{Araneus ventricosus}.
First, the glue balls were prepared in two ways. One was rapid freezing in liquid nitrogen to
keep water. The other was vacuum drying where water was evaporated from the glue balls. Then,
the two types of samples were mapped their element distribution using the TOF-SIMS. No
differences were found in terms of sodium (Na), potassium (K) or the counter ion chlorine (Cl), and proteins were found to be evenly distributed on the surface of the glue ball (Fig. 2a, c). Here, the distribution of proteins were assigned indirectly by the CN⁻ ions, because TOF-SIMS cannot detect intact protein molecules. Next, the glue balls were dissected (Fig. S4) with a focused ion beam (FIB) equipped in the TOF-SIMS apparatus and the element distribution of their cross section was mapped. Na, K, Cl, and the protein were also evenly distributed in the cross section (Fig. 2b, d). However, in case of the cross section analysis, the contrast on the edges and the raised portions were relatively strong due to so-called “edge effect”, a kind of artefact in TOF-SIMS mapping. In addition, we were surprised to find water on the surface of glue ball only under freezing conditions. Here, water was detected in the form of K(H₂O)⁺ because K is sputtered and easily ionized by ion bombardment, therefore K(H₂O)⁺ molecular ion was detected sensitively compared with H₂O⁺. The inside of the glue ball did not contain water. The amount of water on the outermost layer was extremely small and was most likely water vapor that had been attached to the outermost layer of the air before freezing. Although the glue substance coated on the spiral lines formed equally spaced balls by self-organization, the spiral line between the ball and ball was evenly wrapped by the glue substance, similar to a "core-clad" structure (Fig. S1). We were surprised to find that even after immersion in liquid nitrogen, this structure retained a good elasticity. If the glue substance had contained a large amount of water, it would have become brittle after freezing and most likely fractured like an icicle. This further supported our hypothesis that the glue substance did not contain water.

Next, the glue balls were mounted on an aluminum substrate and were left to stand for 10 days in order to come into full contact with the substrate surface (Fig. S5). The glue balls then divided themselves into several regions to form a "fried egg" structure: a center composed of proteins linked to the silk, and an outer ring composed of phosphate (Fig. 3). The chlorine was found to unexpectedly spread to the substrate plane as opposed to the phosphate regions (Fig. 3).
This phase separation of the supersaturated state after stimulation provides chemical evidence for Opell et al.’s suggested glue ball model. According to the Raman spectroscopy data obtained by Amarpuri et al., the intensity of phenylalanine is high in the outermost region (corresponding to the phosphate region in this report) of the glue ball attached to the substrate, whereas the intensity of phenylalanine in other layers is very low. However, the roles of phosphate and phenylalanine are still not clear. They most likely form a supramolecule and may induce mechanochemistry, but this requires further study. There is also a possibility that phosphorus is transformed from a difficult into an easy ionization form. In addition, the measurements of the stretching of the glue ball attached to the substrate indicated that the glue behaves like a viscoelastic solid. It is noteworthy that contact to the substrate alone led to such large changes in properties and structure of the glue ball.

In order to confirm the phenomenon caused by the mechanical force applied to the web after coming into contact with an object, we proceeded with the following experiment. First, the glue balls fixed on the substrate were irradiated with ultrasonic waves (28 kHz). The count number of phosphorus increased significantly (about 3~8 times) after ultrasonic irradiation (Fig. 4). As they were not left to stand for a long time, the glue balls attached to the substrate did not form a "fried egg" structure.

Next, we fired palladium nanoparticles (100 nm) at the orb-web to mimic flying prey. Phosphorus appeared in the places on the glue ball hit by the nanoparticles (Fig. 5a). Next, we placed a live ant on the orb-web (Fig. S6). Varying degrees of phosphorus accumulation were found at the places on the web that came in contact with the ant (Fig. 5b).

Our results confirmed that intact glue balls from orb-webs contained little water, and that the salts and proteins found in the web were present throughout the glue ball. However, contact with an object resulted in the separation of salts and proteins. When the glue ball adhered to the substrate and reached a steady state, it formed a "fried egg" structure (Fig. S7): a region in
center of the protein that loses salt, and an outer ring region composed of phosphate and phenylalanine. The count number of phosphorus was significantly increased after irradiating the glue ball by ultrasonic waves. Regions of the glue ball touched by an object also exhibited changes in phosphorus accumulation. These results suggest that separation and aggregation of phosphorus occurs as a result of mechanical force. There are reports that suggest that the glycoprotein activator is salt $^{18,27}$. The glycoprotein of the glue ball should thus collapse and harden after losing salt $^{27}$. Here, we hypothesized that the glue ball automatically loses salt after coming into contact with an object, whereby the salt is lost from the glue ball and instead accumulates on the object, leading to hardening of the glycoprotein. The substrate could be seen as a "huge prey", whereby the glue ball structures adapt after coming into contact with the substrate in an attempt to fix it to the web until it reaches its limits, resulting in the aforementioned "fried egg" structure. The action of the glue ball in response to a fixed prey effectively prevents the prey from escaping. Our results suggest that the glue ball is a versatile, natural "intelligent" glue that responds to mechanical forces. The mechanisms leading to this versatility should be the subject of future studies. These results provide a basis for the development of new materials and study of supramolecular and mechanochemistry.

Furthermore, from another viewpoint, the radioactive cesium due to the Fukushima Daiichi nuclear disaster may exist in glue balls rich in the same alkali metal potassium. We will investigate the environmental dynamics of cesium via the glue ball in the future plan.

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Supporting Information

Supporting Information showed the following. Schematic of self-organization of the glue ball; Procedure for rapid freezing; Schematic of the process of shooting nanoparticles at the glue balls; Secondary electron images of the glue ball shaved with a Gallium Focused Ion Beam (Ga-FIB); Secondary electron images before and after adhesion of the substrate to the glue ball; Schematic of the "fried egg" structure after adherence of the glue ball on the substrate. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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Figure Captions

Fig. 1. Photograph of the sample. a, Spider (*Araneus ventricosus*). b, Natural orb-web. c, Collected orb-web.

Fig. 2. Secondary electron images and mapping information of the elements in the glue ball. a, Original glue ball under rapid freezing. b, Cross section of the glue ball under rapid freezing. c, Original glue ball under vacuum drying. d, Cross section of the glue ball under vacuum drying. All images are in pseudo-color.

Fig. 3. Secondary electron images and mapping information of the contained elements of the glue ball attached to the substrate. All images are in pseudo-color.

Fig. 4. Secondary electron images and mapping information of the contained elements in the glue ball attached to the substrate before and after ultrasonic irradiation. a, Before ultrasonic irradiation. b, After ultrasonic irradiation. c, Relative count number of phosphate before and after ultrasonic irradiation by normalizing the count number of the aluminum substrate. All images are in pseudo-color.

Fig. 5. Effect of mechanical contact on the glue ball. a, Secondary electron images and mapping information of the contained elements of a glue ball that was hit by nanoparticles. b, Secondary electron images and mapping information of the contained elements of glue balls touched by the ant’s claw. All images are in pseudo-color.
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