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Changes of Calcium Distribution in Glue Ball of Spider's Orb-web under Low-temperature Stress

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The viscous material on the spider web is a special material, called glue ball. It is not a simple adhesive mechanism. This sticky substance will transform into a solidified substance after subjected to mechanical stimulation. Any mechanical stimulus, including touch, will change its elements distribution. In the previous study, the distribution of monovalent metals, hydrochloride, and phosphate in the glue ball was reported. Phosphate groups are considered that play a key role in the versatility of the glue ball. Here we report the distribution characteristics of divalent metal calcium in the glue ball. The calcium may be a key element for low-temperature sensing of the glue ball.

1. Instruction

Among spiders, the most common and well-known species is the orb-weaver spider, which is known to capture its prey by weaving a sticky, "invisible" orb-web. The viscous material appears spherical on the spiral line of the spider web and is called “glue ball”. For a long time, it was believed that the sticky substance of the orb-web is a simple adhesive. However, recently, researchers have discovered that this substance may in fact be an “intelligent” glue material [1], wherein the high-speed impact of prey causes its viscosity to surge. This sticky substance then transforms into a solidified substance that prevents the prey from breaking free [1]. This process relies solely on mechanical stimulation [2].

Based on the currently available information, we know that the main components of this viscous substance are glycoproteins [3–5] and salts [6–14]. The denaturation of glycoproteins after salt loss has been confirmed [2,13,15], suggesting that the salt may play an important role in the versatility of this substance. The distribution of salt in glue ball occurred significant changes after subjecting to various mechanical stimuli [2]. The occurrence of phase separation when glue ball was subjected to mechanical stimulation, especially after coming into contact with an object [2]. After an impact, induced phase separation leads to the loss of salt, which in turn leads to protein denaturation [2]. This suggests that the main function of salt in the web is to change the substance from a viscous material to one that fixes the prey to the web.

Here, we used a lab-made time-of-flight secondary ion mass spectrometry (TOF-SIMS) microscopy to perform a full-scale mapping of the glue ball. By comparing the distribution of the monovalent metal potassium, phosphate group, and the divalent metal calcium, we found that calcium may be a key element for low-temperature sensing of the glue ball.

2. Method

Samples were obtained from the orb-web of the spider *Araneus ventricosus* in the summer night, on a road along Kawaguchi River in Hachioji, Tokyo. The element distribution of the glue balls was observed by using a lab-made TOF-SIMS with a high lateral resolution. See the reference [2] for specific methods.

3. Results and Discussion

The composition of the salt in the glue ball is mainly Na, K, Cl, and phosphate group [2]. Here we compare

the distribution of monovalent metal potassium and divalent metal calcium in the glue ball. Figure 1 shows the distribution of potassium and calcium on the surface and inside of the glue ball after vacuum drying and rapid freezing. Potassium is uniformly distributed from the surface to the inside of the glue ball. This is consistent with the previous report [2]. However, the distribution of calcium was significantly different from monovalent metals. Under vacuum drying, calcium exhibits a nanoparticle-like distribution, both on the surface and inside of the glue ball (Figs.1(a) and (b)). However, under rapid freezing (Figs.1(c) and (d)), the distribution of calcium exhibit less correlation with the shape of the glue ball. The calcium signal that from other than the glue ball was originated in the substrate surface. Here, the intensities of secondary ions are affected by the differences in the type and composition of the material (matrix) due to so-called matrix effect. Therefore, the contrast of the image does not represent the quantitative concentration.

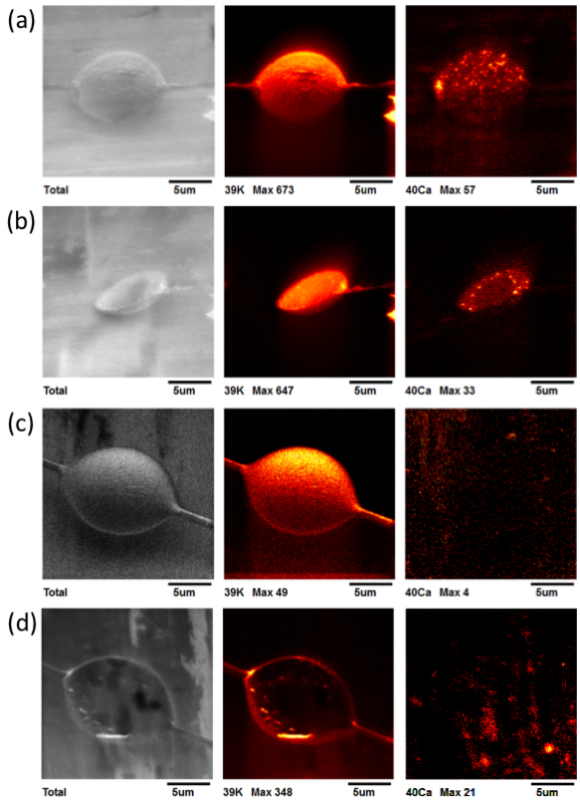

Fig. 1 The mapping information of all elements, potassium, and calcium in the glue ball. (a) Original glue ball under vacuum drying at room temperature. (b) Cross section of (a). (c) Original glue ball under rapid freezing in liquid nitrogen. (d) Cross section of (c). In (b) and (d), the glue balls were dissected with a focused ion beam (FIB) equipped in the TOF-SIMS apparatus.

Glue balls are said to contain little water and remain soft even in soaked liquid nitrogen [2]. It suggests that the glue ball does not form a crystalline structure also in a low-temperature environment. We speculate that calcium ions are present in the glue ball as nanoparticles at room temperature. However, in low-temperature stress, calcium may play a key role in preventing the crystallization of the gel ball. It may cause calcium to become a form that is difficult to be detected as Ca^+ secondary ions.

4. Conclusions

The results of changes in calcium distribution under low-temperatures suggest that it may be involved antifreeze mechanism of the glue ball. However, we are currently unable to determine the form of calcium. The elucidation of the mechanism is our future work.

5. References

[1] V. Sahni, T. A. Blackledge, and A. Dhinojwala, *Nat. Commun.* **1**, 19 (2010).
[2] Y. Zhao, M. Morita, and T. Sakamoto, *Anal. Sci.* **35,** 645 (2019).
[3] E. K. Tillinghast, *Naturwissenschaften* **68**, 526 (1981).
[4] O. Choresh, B. Bayarmagnai, and R. V Lewis, *Biomacromolecules* **10**, 2852 (2009).
[5] M. A. Collin, T. H. Clarke, N. A. Ayoub, and C. Y. Hayashi, *Sci. Rep.* **6**, 21589 (2016).
[6] H. Schildknecht, P. Kunzelmann, D. Krauß, and C. Kuhn, *Naturwissenschaften* **59**, 98 (1972).
[7] H. Schildknecht, *Angew. Chem., Int. Ed.* **15**, 214 (1976).
[8] E. K. Tillinghast, S. F. Chase, and M. A. Townley, *J. Insect Physiol.* **30**, 591 (1984).
[9] E. K. Tillinghast and T. Christenson, *J. Arachnol.* **12**, 69 (1984).
[10] E. K. Tillinghast, *Insect Biochem.* **14**, 115 (1984).
[11] F. Vollrath, W. J. Fairbrother, R. J. P. Williams, E. K. Tillinghast, D. T. Bernstein, K. S. Gallagher, and M. A. Townley, *Nature* **345**, 526 (1990).
[12] F. Vollrath and E. K. Tillinghast, *Naturwissenschaften* **78**, 557 (1991).
[13] M. A. Townley, D. T. Bernstein, K. S. Gallagher, and E. K. Tillinghast, *J. Exp. Zool.* **259**, 154 (1991).
[14] G. Amarpuri, V. Chaurasia, D. Jain, T. A. Blackledge, and A. Dhinojwala, *Sci. Rep.* **5**, 9030 (2015).
[15] V. Sahni, T. Miyoshi, K. Chen, D. Jain, S. J. Blamires, T. A. Blackledge, and A. Dhinojwala, *Biomacromolecules* **15**, 1225 (2014).