Adhesive Substance of *Gasterosteus aculeatus aculeatus*

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An adhesive substance secreted by *Gasterosteus aculeatus aculeatus* was characterized with some carbohydrate tests and SDS-polyacrylamide gel electrophoresis. The results indicated that the adhesive substance would be a glycoprotein or a proteoglycan containing disulfide bonds.

Since an adhesive substance and a fiber produced by an organism have some superior functions such as its intensity and biodegradability, there is great interest in its function. They are also expected to be applied to a novel adhesive and fiber. It has been studied about some adhesive substances produced by marine organisms such as the mussel, *Mytilus edulis*,[1] a diatom,[2] and an oyster,[3] as well as about a spider silk fiber.[4, 5] Waite reported that the adhesive substance of *M. edulis* was a protein containing 3,4-dihydroxyphenylalanine.[1] It is known that the adhesive substance of a diatom consists of acidic polysaccharides[2] and that of an oyster acidic polysaccharides, glycoproteins, and aromatic amino acid-rich proteins.[3] The spider major dragline silk is composed of a fibrous protein.[5, 6] However, there is no information on an adhesive substance of fish.

Gasterosteus aculeatus aculeatus, a diadromous stickleback, is widely distributed on the coasts of north Japan.[7] Male *G. aculeatus aculeatus* secretes an adhesive substance from the kidney to build a conical nest.[7] The adhesive substance has never characterized yet. In this paper, we report about the characteristics of the adhesive substance from *G. aculeatus aculeatus* of Kitakata lake in Japan.

The nests of *G. aculeatus aculeatus* were kindly provided from Echizen matsushima aquarium. The adhesive substance was carefully collected from the nests and washed with distilled water, and then used for the experiment. The adhesive substance was white-colored and insoluble in water. SEM observation of the adhesive substance showed that it was of fibrous (Figure 1).

Figure 1. SEM observation of the adhesive substance of *G. aculeatus aculeatus*.

Table 1. Carbohydrate tests of the adhesive substance of *G. aculeatus aculeatus*

Molish test	positive
Bial test	positive
Chromotropic acid reaction	positive
Seliwanoff reaction	negative
Sulfuric acid-carbazole reaction	negative

First we tested whether the adhesive substrate of *G. aculeatus aculeatus* contains carbohydrates. Table 1 shows the result of some carbohydrate tests. Molish test was positive, proving that the adhesive substance contains carbohydrates. Bial test and chromotropic acid reaction showed that the adhesive substance contains pentose and hexose. The results of Seliwanoff and sulfuric acid-carbazole reaction indicated that the adhesive substance doesn't contain ketose and hexuronic acid.

Next we examined for the existence of amino acids. The adhesive substance was hydrolyzed with 1N sulfuric acid at 373 K for 3-6 h, neutralized with barium hydroxide and then filtered. The concentrated filtrate was applied to paper chromatography (developing solvent: 1-butanol, acetic acid, water = 4, 1, 2, v/v) and detected by the ninhydrin reaction. The result of paper chromatography showed that the adhesive substance contains amino acids (data not shown).

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was done on a 20% polyacrylamide gel by the method of Laemmli under reducing conditions.[8] The adhesive substance gave many bands from 7.6 to 80 kDa in Coomassie Brilliant Blue R-250 (CBB) and the periodate-Schiff (PAS) stainings (Figure 2), indicating that the adhesive substance contains glycoproteins. The heterogeneity in SDS-PAGE of the adhesive substance might be due to the differences in the molecular weight of the polypeptide

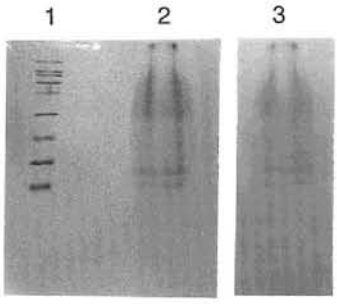

Figure 2. SDS-PAGE of the adhesive substance of *G. aculeatus aculeatus*. A 20% polyacrylamide gel was used. After electrophoresis, proteins in the gel were stained with CBB (lanes 1 and 2) or PAS reagent (lane 3). Lane 1, molecular mass markers (200, 116.2, 97.4, 66.2, 45, 31, 21.5, 14.4, 6.5 kDa); lanes 2 and 3, the adhesive substance.

and/or the glycochain portions. In the preparation of the SDS-PAGE sample, the adhesive substance was insoluble without 2-mercaptoethanol. This result suggested that the glycoprotein of the adhesive substance would have cross-linkages by disulfide bonds.

We tried to solubilize the adhesive substance for the purification. The adhesive substance was insoluble in 1 mol dm^{-3} (M) ethylenediaminetetraacetic acid, 2% SDS, 2% Triton X-100, 8 M lithium perchlorate, 8 M guanidine hydrochloride and 8 M urea; it was soluble in 1 M potassium hydroxide and 88% formic acid. Moreover, the adhesive substance was insoluble in 1.7 M acetic acid but soluble in 100% acetic acid, suggesting that this solubility in acetic acid is similar to that of a mucoid, that is, a glycoprotein. Thus, the adhesive substance could be solubilized with an alkaline solution or an excess acid.

From the results described above, we concluded that the adhesive substance would consist of a glycoprotein or a proteoglycan having cross-linkages by disulfide bonds.

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