Structure, Formation, and Darkening Process of Albedo-reducing Material (Cryoconite) on a Himalayan Glacier: A Granular Algal Mat Growing on the Glacier

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Abstract
Dark-colored material (cryoconite) covering Himalayan glaciers has been reported to greatly accelerate glacier-melting by reducing surface albedo. Structure, formation, and the darkening process of the cryoconite on a Himalayan glacier were analyzed. The cryoconite was revealed to be a stromatolite-like algal mat, a product of microbial activity on the glacier. The granular algal mat contains filamentous blue-green algae (cyanobacteria) and bacteria, and grows on the ice by trapping mineral and organic particles. This structure seems to enable high algal production in nutrient poor glacial meltwater by gathering and keeping nutrient rich particles inside. The dark coloration of the mats promotes melt-hole formation on the ice (cryoconite holes), providing a semistagnant aquatic habitat for various algae and animals in the glacier. Optical and chemical analyses of the cryoconite strongly suggests that their high light-absorbency (dark coloration) is mainly due to dark-colored humic substances, residues from bacterial decomposition of the algal products and other organic matter. Our results strongly suggest that biological activity on the glacier substantially affects the albedo of the glacier surface. The structure of the algal mat seems to be important in the glacier ecosystem and biological process affecting glacier albedo.

Introduction
Impurities in snow and ice, such as airborne particles, have been shown to affect the solar heat-intake to glacier, sea ice, and seasonal snow cover (e.g., Warren and Wiscombe, 1980; Warren, 1982). Dark-colored material with snow and ice can reduce surface albedo (reflection rate) particularly in the visible wavelengths, and thus could accelerate melting. Therefore, the albedo-reducing material in snow and ice can largely affect surface energy budget of snow and ice, and thus possibly affect variation of global cryosphere extent.

Previous studies on albedo reducing impurities in snow and ice, however, have been directed primarily toward airborne particles such as soot, volcanic ash, and desert dust, while microorganisms growing in snow and ice have received virtually no attention (Warren and Wiscombe, 1980; Warren, 1982), probably because biological activity in snow and ice has been believed to be extremely limited. However, many kinds of microorganisms, such as snow algae and bacteria, grow on glaciers and have been reported from various parts of the world (Kol, 1942; Gerdel and Drouet, 1960; Kol and Flint, 1968; Kol, 1969; Kol and Peterson, 1976; Wharton et al., 1981; Kohshima, 1984a, 1984b, 1987a, 1989; Ling and Seppelt, 1993; Yoshimura et al., 1997). The material containing these microorganisms on the glacier surface has been known as cryoconite, which is first named by the arctic explorer, A. E. Nordenskjöld (1875). On Yala Glacier in Langtang region of Nepal, the surface of the ablation area was covered with cryoconite containing a large amount of algae and bacteria. It was reported that the cryoconite substantially decreased the albedo of the ablation area and accelerated surface melting (Kohshima et al., 1993). The albedo of the intact surfaces bearing the cryoconite was substantially lower than that of the surface from which the cryoconite was artificially removed (5% versus 37%). The melting rates of the intact surfaces were reported to be three times larger than that of the surfaces without the cryoconite. Large quantity of the algae and bacteria contained in the cryoconite suggests that these microbes play important roles in the formation process of the albedo-reducing material on the glacier. However, biological characteristics of the cryoconite and intervention of cryomicrobial activity in its formation process were still unclear. Furthermore, although high light-absorbency (dark-coloration) of the cryoconite is important for albedo reduction of the glacier surface, the darkening process of the cryoconite is unknown.

This study aims to clarify the structure, formation, and darkening process of the cryoconite covering Yala Glacier in Nepal Himalayas. The composition and structure of the cryoconite on the glacier surface are described. To confirm the contribution of the biological activity to formation of the material, a simple field experiment was conducted. Also, to clarify the reason for the high light-absorbency of the cryoconite, spectral albedo measurement and humic substance analysis of the cryoconite were conducted. Ecological implications of the cryoconite structure and albedo-reducing effect by the biological activity on the glacier surface are discussed.

Study Site
The research was carried out at Yala Glacier (5100–5750 m a.s.l.), Langtang region, Central Nepal (Fig. 1), between July
Sampling and Analysis

The cryoconite and the surface ice beneath it (1-2 cm in depth) were collected with an ice axe in the ablation area (5150 m a.s.l.). The collected samples were melted and kept in 100-ml clean polyethylene bottles and preserved as 3% formalin solution.

Windblown material, which is the original source of the cryoconite, was collected from new snow on the glacier (at 5150 m a.s.l., 20 May 1996). The windblown material comes from ice-free ground surfaces surrounding the glacier in premonsoon dry season (early spring). Since the collection of the windblown material was done before melting season, the material can be considered not to be affected by any biotic activity on the glacier. The windblown material was analyzed in the same way as the cryoconite described blow.

The amount of organic matter in the cryoconite was measured by the following method. After the samples were dried (65°C, 24 h) and weighed, they were combusted for 1 h at 1000°C in an electric furnace. Percentage of weight reduction by this procedure was measured. Carbon and nitrogen content (mass) of the cryoconite was measured with a NC analyzer (S umigraph-80, Shimadzu Simazu Co.).

The structure of the cryoconite was observed with an optical microscope, a fluorescent microscope (Nikon Optiphot 2), and a scanning electron microscope (FE-SEM, S-800, HITACHI Co.). DAPI (4',6-diamidino-2-phenylindole) staining was used for bacteria observation with the fluorescence microscope. For scanning electron microscopy, the material was dried with a critical point dryer (HC-80, HITACHI Co.) and was coated with carbon and Pt-Pd after drying.

To observe inner structures of the cryoconite, thin sections were made. The samples were dehydrated in a series of ethanol and acetone (50%, 70%, 80%, 100%, 100% of ethanol, and 100%, 100% of acetone), and then embedded in polyester resin. The embedded sample was ground by a grinder with abrasive to a thin section (approximately 0.1 mm thickness). The section samples were observed with an optical microscope.

To examine whether the structure of the cryoconite is formed by algal growth, the following simple experiment was carried out on the glacier. The cryoconite was ground up into fine particles with a pestle and a mortar (2.5 ml in volume), then stored in two
screw-capped 200 ml transparent plastic pots with 30 ml of meltwater. In order to inhibit algal growth, 1 ml of 80% CuSO₄ solution was added into one of these pots. The pots were set on the glacier ice in the ablation area (5220 m a.s.l.) for 4 mo during the monsoon season (from 25 May to 30 September 1996). Then, the structure of the cryoconite in the pots was observed with a microscope.

To analyze optical characteristics of the cryoconite, the spectral albedo (light reflectance) was measured. The samples were ground up into fine particles with a pestle and mortar and collected on Millipore filters (HAWP047XX, pore size: 0.45 μm) by filtering the sample waters. The amount of material collected on a filter was approximately 10 mg cm⁻² in dry weight, which was enough to completely cover the filter surface with the cryoconite. The reflected irradiance of the cryoconite on the filter was measured by a spectrometer (FieldSpec FR, Analytical Spectral Device Inc.) with a light source of integrating sphere (USC-1200V, LabSphere co.) just after filtering, at 1-nm intervals in visible wavelengths from 350 nm to 950 nm. The spectral albedo was calculated from the reflected irradiance of the sample and that of a standard white reference plate.

The surface albedo of clean bare ice and bare ice with cryoconite was measured on 30 September 1996, by a portable photometer (model 2703, Abe Sekkei co.) at 5220 m a.s.l. in ablation area, where the surface is almost horizontally flat. The bare ice without cryoconite is set on the glacier surface by artificially removing the cryoconite. The measured wavelengths were 400, 450, 500, 550, 550, 600, 650, 700, 750, 850, and 950 nm. The albedo was calculated from the total of reflected irradiance of the surface and that of a standard white reference plate. The mean albedo was obtained from values of five different surfaces.

Extraction and measurement of humic substances contained in the cryoconite was conducted by Nagoya method (Kumada, 1985). The extracted substances were quantified titrimetrically with 0.1 N KMnO₄.

**Figure 3.** The cryoconite on Yala Glacier. The cryoconite consisted mainly of small dark-colored granules (cryoconite granules). Scale bar, 5.0 mm.

**Results and Discussion**

**COMPOSITION AND STRUCTURE OF THE CRYOCONITE**

The cryoconite mainly consisted of small dark-colored granules (50–60% of the total volume, Fig. 3). The granule is referred to as cryoconite granule below. The size of the granules ranged from 0.1 to 3.0 mm (mean = 0.5 mm, SD = 0.2) in diameter. The granules were soft and easily crushed between fingers, but were strong enough to maintain their shape in rapidly flowing water. Microscopy of the granules disassembled by ultrasonication revealed that they were aggregations of filamentous blue-green algae (filamentous cyanobacteria, two species of Oscillatoriaeae algae), unidentified coccolid blue-green algae (coccolid cyanobacteria, 4 ± 0.5 μm in diameter, Chroococcaceae alga), bacteria, amorphous black matter, and mineral particles. In addition to the granules, the cryoconite contained transparent and pale brown mineral particles (30–40% and 10–20% in volume, respectively), and organic particles including unicellular snow algal cells (<1% in volume). The dark coloration of the material seemed mainly due to the granules because the other particles were pale and small (<0.1 mm in median diameter).

In contrast, the windblown material mainly consisted of mainly brown or transparent mineral particles, and some plant fragments. The cryoconite granules were not observed in the windblown material, suggesting that the cryoconite granules are formed on the glacier surface. Measurement of organic matter content revealed that the windblown material contained significantly less organic matter than the cryoconite. The cryoconite contained 7.8% (range: 5.4–9.9%) organic matter in dry weight, whereas the windblown material contained 1.8% organic matter. The carbon contents (measured by a NC analyzer) in the cryoconite and the windblown material were 2.7 and 0.55%, respectively. The nitrogen contents were 0.27 and 0.038%, respectively. The larger content of organic matter in the cryoconite relative to the windblown material suggests that organic matter in the cryoconite was increased considerably by algal photosynthesis on the glacier.

Figure 4 shows the surface and cross section of the cryoconite granule observed by fluorescent microscopy. Autofluorescing algal pigments are viewed as red color. The surface of the granule appeared to be densely covered with filamentous blue-green algae (Fig. 4a). Observation of the autofluorescent algae in the cross section of the granule revealed that the algae were distributed in a thin layer at the surface of the granule (30–100 μm in thickness, Fig. 4b). The inner part of the granule must be composed of nonfluorescent materials, such as dead algae (i.e., without chlorophyll a autofluorescence), amorphous black matter, and mineral particles. Figure 5 shows the thin section of the granule observed with an optical microscope. Inside the granule, there were abundant transparent or pale brown mineral particles (0.1–0.5 mm in diameter) surrounded by amorphous black matter. Figure 6 shows the disassembled granule observed with a fluorescent microscope. DAPI stained bacteria (blue) were observed to attach to algal filaments and/or the amorphous matter. Figure 7 shows the green-blue algae and bacteria observed with a scanning electron microscope. The spherical or cylindrical cells of bacteria were observed on the surface of filamentous blue-green algae (Fig. 7a). The bacteria cells were often covered with mucus-like substance (Fig. 7b). These bacteria were observed at both the surface and inside the granules. The mucus-like substance and the amorphous black matter observed by microscopy were thought to be algal and bacterial mucilage and/or decomposing organic matter.

These observations of the granule structure strongly suggest that the granule is a mat formed by the filamentous blue-green...
algaes. The granules apparently enlarge as filamentous blue-green algaes grow and trap particles on their surface. The amorphous black matter observed in the granules may be a product of bacterial decomposition of old algae and other organic particles trapped in the granules. The spherical shape of the granules seems to be maintained by the entanglement of algal filaments and adhesive substances around the mineral or organic particles.

FORMATION OF THE CRYOCONITE BY ALGAL GROWTH

Figure 8 shows the result of the simple field experiment to examine whether the structure of the cryoconite is formed by algal growth. At the end of September, a sheet of algal mat (430 $\pm$ 69 $\mu$m (SE) in thickness, n = 39) had formed in the pot without CuSO$_4$ only (Fig. 8a). The sheet was attached to the flat bottom of the pot, and autofluorescing algal pigments revealed that its surface consisted of densely filamentous blue-green algae (Fig. 8b). In the cross section of the mat, the structure was strikingly similar to that of the cryoconite granule. In contrast, in the pot with CuSO$_4$, the particles remained loose and were easily dispersed by movement of water (Fig. 8a). No algal growth was observed in this pot. The result implies that an algal mat can be formed on the glacier by the growth of filamentous blue-green algae. The spherical shape of the cryoconite granule is probably due to physical shaping forces by meltwater movements and absence of large and stable substrata for algal growth.

The thickness of the algal mat formed during the experiment is likely to show the annual growth of the algal mat on the glacier. The experiment period (from May to September) is the

**FIGURE 4.** a. The surface of the cryoconite granule observed by a fluorescent microscope (red: algal pigment autofluorescence). Scale bar, 0.5 mm. b. The cross section of the cryoconite granule observed by a fluorescent microscope (red: algal pigment autofluorescence). Scale bar, 0.5 mm.

**FIGURE 5.** The thin section of the granule observed with an optical microscope. Scale bar, 0.5 mm.

**FIGURE 6.** Fluorescent microscopy of ultrasonicated cryoconite granule. Bacteria attached to a filamentous blue-green alga were observed. (blue: DAPI stained bacteria, red: filamentous blue-green algae with algal pigment autofluorescence) Scale bar, 5 $\mu$m.
only season when the algae can grow on the glacier, because in winter (from October to April), the glacier surface is covered with thick snow (more than 2 m, Iida et al. [1987]) that prevents photosynthesis of algae. In the cross section of the cryoconite granule, obscure concentric layers were observed (Fig. 5). This layer structure was observed in 30 out of 40 granules. The thickness of the outer layers was roughly the same as the thickness of the algal mat formed in the pot (406 ± 32 μm versus 430 ± 69 μm, mean ± standard error). This suggests that the layers of the cryoconite granule are annually formed by algal growth in summer. Thus, the growth rate of the granules can be estimated to be approximately 400 μm per year. In some large granules (>1.5 mm in diameter), a few smaller granules were observed in their cross section. This structure implies that these granules had enlarged by fusion of a few smaller granules. Large-sized granules over 3 mm in diameter were very rare, and those of 2 to 3 mm in diameter were often partly split, suggesting that the maximum size of the granules is about 3 mm in diameter. Size is probably limited by the binding ability of the algal filaments and organic matter that maintain the spherical shape of the granule.

The layer structure and formation process of the cryoconite granule well resemble stromatolites. Stromatolites are structures formed by sediment trapping and binding within microbial communities termed algal mats (Walter, 1976). They have been found to live in extreme environments, such as high saline waters, hot springs, and permanent frozen lakes. The glacier, which is an extremely cold environment for life, is also considered one of the habitats of modern living stromatolites.

DARKENING PROCESS OF THE CRYOCONITE

Figure 9a shows the spectral albedo of the cryoconite in the visible region, the mineral particles contained within the cryoconite (extracted from the cryoconite by removing the organic components with heated hydrogen peroxide), and the windblown material. The spectral albedo of the cryoconite was constantly low in the 350- to 950-nm wavelengths region (mean = 6.5%), indicating blackish coloration (high light-absorbency). Figure 9b shows the spectral albedo of clean bare ice and bare ice with cryoconite. The spectral albedo of the bare ice with cryoconite was approximately 20 to 40% lower than that of the clean bare ice, and was almost constant in the wavelengths region, corresponding with the spectrum of the cryoconite. This indicates that the surface albedo is substantially reduced by the high light-absorbency of the cryoconite.

In the spectrum of the cryoconite, no obvious absorption by algal pigments was observed, suggesting that the high light-absorbency is not due to algal color itself. Bacteria, sparsely distributed on surface of the granules, also cannot be a major cause of the high light-absorbency. The spectral albedo of the mineral particles (mean = 22.3%) was higher than that of the cryoconite for all wavelengths (Fig. 9a). The mineral particles appeared pale brown. This result indicates that the high light-absorbency of the cryoconite is mainly due to the organic components, especially the dark-colored amorphous organic matter observed by optical microscopy. The spectral albedo of the windblown material (mean = 15.8%) was also higher than that of the cryoconite, indicating that light-absorbency of the cryoconite increased after the material was deposited on the glacier surface.
Extraction of humic substances from the cryoconite revealed that the cryoconite contained a considerable amount of dark-colored humic substances: 1 g of the material contained 7.0 ml (amount of 0.1 N KMnO₄ consumed) of humic acid. This amount was equivalent to approximately 11% of total carbon in the cryoconite. The result suggests that the high light-absorbency of the cryoconite is due to the dark-colored humic substances, similarly to dark coloration of soil organic matter. Humic substances are thought to be highly polymerized compounds of residues remaining after bacterial decomposition of organic matter (Kononova, 1966; Kumada, 1987). Dark coloration of the humic substance is due to a large amount of conjugated molecular bonds forming a part of the compounds (e.g., benzene ring, polyethylene chain), which can effectively absorb wide range wavelength of visible light. The humic substances extracted from the cryoconite are likely to originate from bacterial decomposition of algal products and/or other organic particles in the cryoconite.

The C/N ratio by weight of the cryoconite supports the idea that organic matter is decomposed by bacteria on the glacier surface. The C/N ratio of the organic matter in the cryoconite (mean = 10.0 ± 0.1 SE, n = 3) was close to that of well-decomposed soil organic matters (Begon et al., 1990), suggesting that the cryoconite contains much decomposing organic matter. In contrast, the C/N ratio of the windblown material was higher than that of the cryoconite (14.5 versus 10.0). The C/N ratio of little decomposed soil organic matter is generally high (20-50) and decreases with microbial decomposition until near 10 (Begon et al., 1990). Therefore, the lower C/N ratio of the cryoconite relative to the windblown material suggests that bacterial decomposition of organic matter proceeds on the glacier. Bacteria in the cryoconite probably convert algal products and other organic matter into dark-colored humic substances, which effectively increase the light-absorbency of the cryoconite granules by covering pale-colored mineral particles.

**ECOLOGICAL IMPLICATION AND ALBEDO-REDUCING EFFECT OF THE CRYOCONITE**

A microbial assemblage similar to the cryoconite granule has been reported from Antarctic perennial lake ice in the McMurdo Dry Valleys (Priscu et al., 1998). The microbial assemblage found in the liquid-water inclusions that developed in the lake ice in response to solar heating of colian-derived sediment in the ice, also consisted of filamentous blue-green algae, bacteria, and organic- and inorganic-particles. The assemblage was reported to be a complex microbial consortium capable of contemporaneous photosynthesis, N₂ fixation, and decomposition, in extremely nutrient poor conditions. Similarity in microbial composition of the Himalayan granule to the Antarctic assemblage suggests that the granule may also serve as nutrient-enriched microzones that allow microbial propagation in a nutrient-depleted glacial ecosystem. On the glacier, the main nutrient source for the algal growth is thought to be organic and inorganic windblown particles, since the nutrient level of the meltwater is generally low. For example, the concentrations of NO₃⁻ and PO₄³⁻ in the meltwater are 0-0.04 mg L⁻¹ and 0 mg L⁻¹, respectively (0) means not detected. Detection limits are 0.005 and 0.013 mg L⁻¹, respectively), which are significantly lower relative to typical oligotrophic freshwater located at a similar elevation in the same region (Yoshimura et al., 1997). The structure of the cryoconite granule seems to enable high algal production in the nutrient poor glacial meltwater by collecting and storing nutrient-rich particles inside and by recycling the nutrients through bacterial decomposition. Furthermore, the high light-absorbency of the cryoconite promotes melt-hole formation on ice by absorbing solar radiation. These cylindrical water-filled melt-holes, called cryoconite holes (Gerdel and Drout, 1960; Wharton et al., 1981 McIntyre, 1984; Wharton et al., 1985; Takkenchi et al., 2000), serve as nutrient-enriched microzones in the glacier eco-
windblown material consisting of pale-colored mineral particles and plant fragments, into granules with high light-absorbency. Our results suggest that the algae produce organic matter, increasing amount of the impurities, and that bacteria decompose the organic matter, increasing light-absorbency of the impurities. This biotic modification of the impurities is likely to significantly influence the surface albedo of snow and ice at visible wavelengths.

The spectral albedo of the glacier surface (Fig. 9b) indicates that the cryoconite on the surface substantially decreases the surface albedo. The albedo decrease due to the cryoconite has been estimated to be approximately 30%, which is equivalent to 53.4 W m⁻² of heat gain and 13.8 mm d⁻¹ of ablation rate on the glacier surface (Koshima et al., 1993). Without biological activity on the glacier, the material on the glacier surface consists of only windblown material, which consists of a smaller amount of pale-colored mineral particles and plant fragments. The effect of the material on albedo reduction would be reduced compared with that of the cryoconite. The biological effect could be 8 to 15% of albedo reduction based on the difference of the spectrum and organic matter content between the cryoconite and windblown material. The quantitative effect of the biological activity on albedo reduction is still not clear. Theoretical model calculations or in situ experimental measurement of albedo on the glacier is necessary to accurately quantify the effect. However, our results obviously suggest that the biological activity plays an important role in the formation of the cryoconite and in the albedo reduction on the glacier surface, and thus probably affect glacial mass balance and hydrology.

The granular algal structure was found at all of the nine Himalayan glaciers which we have studied (unpublished data). Furthermore, the algal structure has been found on glaciers in other parts of the world, e.g., N.W.T., Canada (Takeuchi et al., 2001), Arctic Norway, and Tibetan Plateau of China (unpublished data). Previous studies of cryoconite holes in Greenland and Antarctica, have found that hole sediments contained filamentous blue-green algae (Gerdel and Drout, 1960; Wharton et al., 1981, 1985), suggesting the existence of similar algal mats in these areas as well. The biological processes affecting glacier albedo, a previously unexpected process in geoscience, seems to be a common phenomenon occurring in many parts of the world.

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