Raphide Crystal Structure in *Agave Tequilana* Determined by X-ray Originating from Synchrotron Radiation

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The first single crystal structure of small natural raphides in an agave plant is completely determined using an intense X-ray originating from a synchrotron radiation. The SEM image shows that the tip of the crystal is approximately hundreds of nanometer in width sharply grow to stick to the tissue of herbivorous vermin. Furthermore, the crystal develops cracks that propagate at an inclination of approximately 45° towards the direction of crystal growth such that the crystal easily splits into small pieces in the tissue.

Most bulbous plants store crystals of calcium oxalate in tissues as biological reserves of calcium ions.[1] Raphide in *Agave tequilana*, which is one such crystal, has a unique external form where both ends are minute, transparent, and needle sharped.[2] In addition, it is serves as defense mechanism that the plant uses against herbivorous vermin that causes harm by feeding on the inulin in flesh tissues.[3] Furthermore, the raphide causes irritant dermatitis in human beings, which is similar to the blisters on the arm by a fish-tail palm.[4] Therefore, we are anxious to clarify the reason behind the irritant activity of the raphides.[5]

Raphides are small crystals whose lengths vary from submicrons to hundreds of microns. Schmidt and Moult showed a photograph wherein a raphide crystal was stuck in the skin of a piglet causing dermatitis.[6] In 1965, Arnott and co-workers showed that raphides have a structure similar to that of whewellite ($CaC_2O_4 \cdot H_2O$) by an XRPD pattern of the small single crystal.[7] However, the experiments conducted by them did not reveal the detailed crystal structure because the raphides obtained from the plant were extremely small microcrystals with a rotational twin.[8] Here, we show the complete crystal structure of a small needle-shaped raphide crystal, which is determined from the data collected using a high intensity X-ray from a synchrotron radiation.

The irritant component in *Agave tequilana* has been identified to be a relatively large raphide.[5] A colorless crystal with the dimensions of $4 \times 5 \times 280\,\mu m^3$, selected from the cleaner raphides, was prepared by cutting both ends of the twin segment. Bragg spots on the crystal were collected at $-73.2\,^\circ C$ under vacuum (5.5×10^{-4} Pa) using synchrotron radiation (8 GeV, $\lambda = 0.61650$ Å) through the Si (311) monochromator. The MAC Science low-temperature vacuum X-ray camera equipped with an imaging plate (IP) area detector is used for the BL02B1 beamline at the SPring-8 facility. The collected data were indexed and the reflections were integrated using DENZO and subsequently scaled using Scalepack.[9] All calculations were performed with the teXsan crystallographic software package.[10] The structure was solved by direct methods (SHELXS-97)[11] and refined for 135 variable parameters with full-matrix least-squares on F^2 for the 938 unique reflections. The crystal data show a space group of monoclinic $P2_1/a$ (No. 14), with lattice parameters $a = 10.090(2)$, $b = 14.549(4)$, $c = 6.270(3)$, $\beta = 109.53(2)$ Å, $V = 867.5(6)$ Å^3, $Z = 8$ and $D_{calcd} = 2.207$ g/cm^3. The results obtained were converged to $R_1 = 7.72\%$ and $R_w = 23.79\%$ ($R = 15.88\%$ for all data), and GOF $= 0.87$. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.242 and $-1.154\,e^-/Å^3$, respectively. Four hydrogen atoms and the oxygen atoms O(15) and O(16) of water were not contained at the refining.

Upon analyzing the crystal data, we have found that the structure of raphides is similar to that of the expected whewellite mineral with a high density of 2.216 g/cm^3.[12] Figure 1 shows the ORTEP view of the raphide crystal obtained by our experiment. Each of the two Ca^{2+} has an octadentate coordination sphere with distorted square antiprisms, which is coordinated by an oxygen atom of water and seven other oxygen atoms originating from a five-membered oxalate. (Coordination distances of Ca^{2+}–O are in the range from 2.407(3) to 2.481(3) Å) An oxalate ligand coordinates to six different Ca^{2+}, where eight lone pairs of the four oxygen atoms are coordinated to the metal ions. The coordination structures of oxalate surrounding Ca^{2+} consist of form A by bridging two Ca^{2+} as two didentate chelates and form B with four Ca^{2+} in the form of a monodentate ligand, as shown in Figure 2. Owing to a complicated three-dimensional network, the direct distances between two Ca^{2+} indicate four different values Ca(1)–Ca(2) = 3.784(1) Å, Ca(1)–Ca(1)^{*1} =

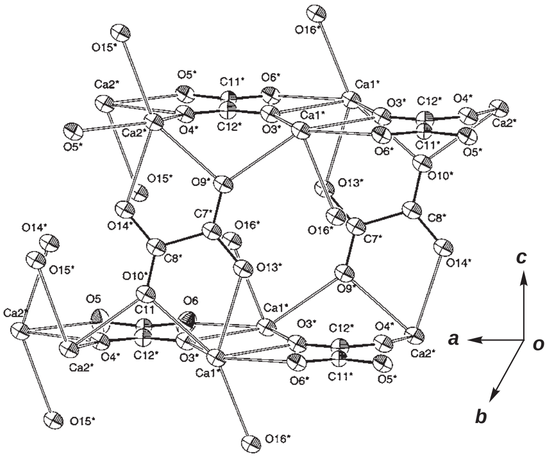

Figure 1. The ORTEP view shows the crystal structure of natural raphide. The numbering schemes of the atoms and their respective lattice axes are superposed on it.

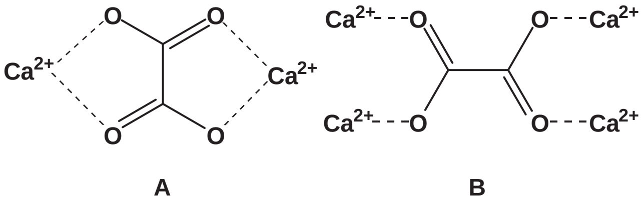

Figure 2. Coordination patterns of Ca^{2+} to oxalate in the raphide: oxalate works as the bridging chelate ligand for two Ca^{2+} (Form A) and as four monodentate ones for four Ca^{2+} (Form B).

3.840(2) Å, Ca(1)–Ca(2)^{*2} = 3.895(1) Å, and Ca(2)–Ca(2)^{*3} = 3.895(1) Å (where, *1: $-x+2$, $-y$, $-z+1$; *2: $x+1/2$, $-y+1/2$, z; *3: $-x+1$, $-y$, $-z+1$). Then, Ca(1) and Ca(2) are bridged by the two oxygen atoms of O(3), O(4), O(5), O(6), O(9), and O(10) (the average angle for Ca–O–Ca is approximately 103.5°) and the two carboxylates of form B in the oxalate. The O(15) and O(16) atoms of water molecules coordinated to Ca(1) and Ca(2), respectively (Ca(1)–O(16) = 2.433(4) and Ca(2)–O(15) = 2.542(4) Å). The O(16) atom of water has a slightly large temperature factor $B_{eq} = 3.2(1)$. Furthermore, the two oxygen atoms O(15) and O(16), that belong to water molecules, would be connected to each other by hydrogen bonds through a proton as in the case of whewellite (O(15)–O(16) = 2.753(5) Å). This experiment could not accurately recover the proton location.

Usually, the crystals of whewellite ($CaC_2O_4 \cdot H_2O$) prepared in the laboratory from gels grow to a colorless and prismatic habit but not a needle one.[13] This is probably one of the most important factors for raphides wherein their crystal growth is periodically controlled to achieve a needle shape with a chiral rotational twin on the crystal surface by contact with certain proteins.[14] Figure 3 shows the scanning electron microscope (SEM) image of the tip of a needle-like raphide crystal. It should be noted that the tip of the crystal measures hundreds of nanometers in width and has sharply grown up to stick into the tissue of herbivorous vermin. Furthermore, the SEM image also shows that the crystal develops cracks that propagate at an inclination of approximately 45° in the direction of crystal growth, so that the crystal can easily split into small pieces in the tissue.[2] Therefore, the raphide with a tip measuring in the submicron range plays an important role in the insertion of an irritant-active potion with proteins in the tissue by penetrating through a cell membrane cuticle.[15]

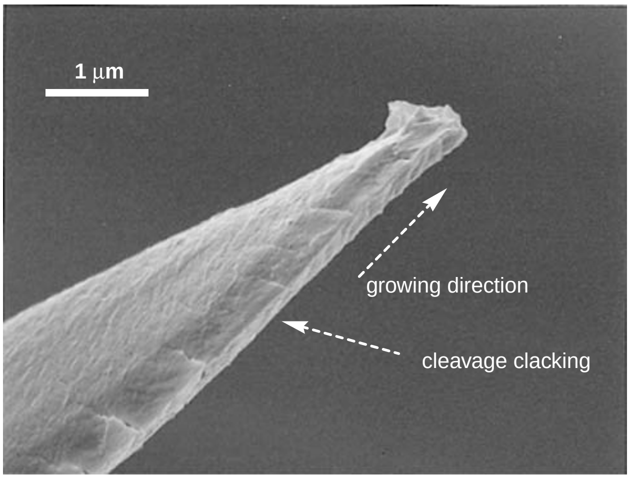

Figure 3. The SEM image on the tip of raphide. The raphide was coated with Pt and Os. (Bar = 1 µm) Note that raphide has the cleavage clacking with an inclination of approximately 45° in the direction of growth of the tip.

In this way, we have, for the first time, demonstrated the crystal structure of raphides by collecting data using the intense X-ray originating from a synchrotron radiation. Such a method would serve as an important procedure for investigating the structure of a micro-ordered biological small crystal that grows in a tissue of the living system.

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