Biomineraliztion of barium at deep-sea hydrothermal systems

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学位論文要旨

ABSTRUCT

Biomineralization studies focused on the deep-sea hydrothermal system in the North Knoll of Iheya Depression, the Middle Okinawa Trough, and found both Gram-negative archaea from the 180, 238 and 311℃ vent sites and bacteria from the 70℃ vent site. Additionally, electron microscopy found barite ($BaSO_4$) particles on the cellular surface, and geochemical investigation indicated that their crystal formation processes and conditions are inconsistent with the relationship between the morphology of abiotic precipitation and saturation state demonstrated by past experiments. Chemical analyses of the hydrothermal fluid samples showed the high Ba concentration(23.2 μ M/kg in maximum), which reflects a typical characteristic of hydrothermal fluids in back-arc basin.

Biomineralization of barium is closely related to the physiological traits of producing inclusion bodies, and transport system associated with the simple cellular fabric with S-layers. Crystal nucleation of $BaSO_4$ both inside and outside the excreted organic molecalar results in forming indeterminate barite particles10 nm in size. Furthermore, it has been indicated by the archaea at the 180℃ vent site that S-layers flexible characteristics of "self-assembly" causes sheaths formation for S-layers, in order to protect the cell from the surrounding environmental stress such as growth temperature. This hypothesis on the archaeal sheaths formation suggests that sheaths formation is possibly renovated from S-layers, due to comparatively fast reaction time in forming crystal nucleation for barite with the excreted SO_4^{2-} and dissolved Ba ion in the hydrothermal fluid. It is followed from this that S-layered structure is flexible in response to their surrounding environment.

INTRODUCTION

Since deep-sea hydrothermal vents were initially discovered, the obligate milieu of marine hydrothermal systems with features such as "black smokers" or "white smokers" has focused on much interest in hydrothermal processes for the explanation of an array of geochemical processes and phenomena.

The inputs of heat and materials from the deep-sea hydrothermal systems also narrate the possible energy for luxuriant microbial communities in the vicinity of deep-sea hydrothermal vents, especially, *chemolithoautotrophic organisms*, unknown to prior to the discovery of venting. This reminds of that large populations of bacteria in/around deep-sea hydrothermal vents mediate primarily the dissolution and precipitation of minerals through their *biomineralization* .

Most prior microbiological studies have made efforts to interpret Fe- and Mn- oxide, Fe-silicate, and sulfide formation associated with microbial activities. However other microbial mineral formation has not much been reported yet. In fact biomineralization of Ba, in terms of oceanology, is of fundamental importance in conditioning chemical and biological fluxes in the oceans, while a large number of geochemical experiments have only focused on the mechanism of barite formation to elucidate the mass transfer mechanism within hydrothermal sites.

Accordingly, in order to find out the mechanism of microbial Ba accumulation and barite formation at biological-mineralogical-geochemical interfaces within a deep-sea hydrothermal system, *in situ* observations by means of electron microscopy, and biological and mineralogical analyses were conducted, using the samples from the four vent sites in the North Knoll of Iheya Depression (NKID), the Middle Okinawa Trough.

HYDROTHERMAL PHENOMENA OF NKID

Okinawa Trough located between the Nanseishoto and the Continent of China is one of the back arc basins, extends from the Ilan Plain in northern Taiwan to the shallow sea southwest of Kyushu. Iheya Depression had been evaluated to be the most active area of submarine volcanism in the Middle Okinawa Trough. At present, the Okinawa Trough is considered to be at the first stage of back-arc spreading, and these activities are related directly to submarine volcanic activities of island-arc rather than a product of submarine volcanic activities caused by spreading of the back-arc basin. In 1996 and 1997, research dives of the "Shinkai 2000" were undertaken at the NKID 950-1040 m deep, and total six active 'clear smoker' vent sites were discovered. The maximum fluid temperature measured during the 1996 and 1997 research dives was 238℃ and 311℃ respectively. Whereas emission situation of the 238℃ fluid is characterized by directly diffuse flow from gentle mounds, the 311℃ fluid was observed discharging

vigorously up to 50 cm from a chimney 3m in height. It is notable that the maximum fluid temperature of 311℃ is nearly equal to boiling point at the depth of the Iheya seafloor hydrothermal systems.

SAMPLES AND METHODS

The samples are divided into two categories; i.e. solid samples and fluid samples. Solid samples are columnar bottom samples of the seafloor sediments, fragments of both inactive and active chimneys, hydrothermally altered rock. Each fluid sample from the 238℃, 180℃, 70℃, and 311℃ vent sites was filtered for chemical analyses, and the filters were used for the studies.

In order to identify the constituent minerals in all solid samples, X-ray powder diffractometer (XRD) was used. Energy dispersive X-ray fluorescence analyzer (ED-XRF), and electron probe micro analyzer (EPMA) were used for determining qualitatively and quantitatively elemental characteristics and the elemental distribution of the rock. To confirm the existence of organisms in the suspended materials on the filters, Fourier transform infrared spectrometer (FT-IR) was used. The micro-morphological traits of all solid samples and the materials suspended in each fluid samples were observed by a scanning electron microscope (SEM), and qualitative and semi-quantitative analyses were carried out by an energy-dispersive X-ray analyzer (EDX) equipped with the SEM. In order to observe further the both inner and outer microtexture of organisms, and the morphological characteristic of very fine materials, Transmission Electron Microscope (TEM) was used. A selected-area electron diffraction patterns were obtained from the selected minerals within the TEM image, to identify such very fine minerals.

RESULTS

1. Mineralogical studies

The results of XRD and SEM-EDX analyses of the fragments of both inactive and active chimneys indicated that, likewise inactive chimneys, the active chimney is composed of sulfides; sphalerite, chalcopyrite, galena, pyrite, and wurtzite, while currently venting "clear smoker". Furthermore, reflections of barite were also detected by XRD from both chimneys fragments. The columnar polyhedral barite minerals 20-30 μm long and 5 μm wide were observed by SEM-EDX in the specimen from the fragment of inactive chimneies. In contrast, the mineral phases present in the hydrothermally altered rock were non-sulfide minerals, and ED-XRF patterns of that the rocks show abundant Ba and S. Backscatttered electron image of the thin-sectioned specimens of the rock showed a large amount of platy and acicular barite 40-120 μm in size. Furthermore, EPMA analysis found that Be content level of the platy part of the

barite is more intense than that of the acicular part. From the SEM-EDX analyses of the suspensions in the fluid samples found variously rectangular barite particles 2-3 μm in size.

2. Microbial studies for planktonic microbes in the fluid samples

The FT-IR spectrum of the clumps from the fluid samples at the 180, 238 and 311℃ vent sites showed the existence of ether bond (C-O-C) which is a specific membrane component of archaea, although the peaks of ether bond were not confirmed from the clumps at the 70℃ vent site.

160 EDX spectra were obtained from the microbes in the fluid samples, and numerical values calculated by setting off the count of detected background against CPS of each peak were plotted in triangular diagram for P, Ba and S for the microbes at the 70, 180 and 238℃ vent sites. The narrow plotted area is attributed that microbial accumulation of Ba is not predominant at the 70℃ vent site. From the triangular diagram for the microbes at the 180℃ vent site, all plots are on a straight line from the apex of P to the base of the diagram. On the other hand, all plots are located to the left side of the diagram of P, Ba and S of the microbes at the 238℃ vent site. The Ba content 0-7% against high content of S(44-52%) have been found from the microbes at the 238℃ vent site(Fig.1).

TEM observation of the microbes in the fluid sample from the 70℃ vent site showed microbial barite accumulation on cellular surface. Rod-shaped microbes covered wholly with thin membrane remain some of the barite particles within the thin membrane. TEM observation revealed a specific barite accumulation pattern on the cellular surface of the microbes in the fluids samples from the 180℃ vent site. A microbe shows that particle distribution described here as "raft foundation". The morphologies of the barite particles are characterized as mainly elliptical crystal, 10.7 - 16.0 nm in major size and some particles 50 nm in size. Further observation showed a microbe covered with barite, which is like a sort of loricate(Fig.2). TEM observation of the microbes in the fluid samples from the 238℃ vent site showed single or several elliptical materials 100-250 nm in size around the cell. High resolution image demonstrated that these elliptical materials serve as "storage" of barite particles15-30 nm in size, which develop independently as a polycrystal. The elliptical materials are entangled with excreted materials, which leads to remaining of the mode of occurrence after released from the cell(Fig.3). TEM observation showed spherical microbes 0.5 μ m in size in the fluid sample from the 311℃ vent site, which have single or a couple of granular particles in the interior of the cell. These spherical microbes possess hair-like materials such as pili, which extend to 300-500 nm long from the cell. Coupled microbes are conjugated mutually with the pili, which are inserted into the interior of the other cell. Sphalerite(ZnS)was observed from the extra-cellular particles.

DISCUSSION

1. Concentration of SO_4^{2-} and Ba^{2+} in the fluid samples

The previous studies had shown that decrease of the SO_4^{2-} concentration from 26.5 mM/kg of seawater to close to zero in hydrothermal fluids. Chemical analysis indicated low concentration of SO_4^{2-}, ranging from 0.5 to 3.6 mM/kg from the fluid sample from the 238℃ vent site, while the high SO_4^{2-} concentration was designated by the fluid samples from the 70, 180 and 311 ℃ vent site, which reach 11.0, 13.2 and 25.3 mM/kg, respectively. These SO_4^{2-} concentrations correspond to 40-90% of the seawater SO_4^{2-} concentration.

The maximum measurement of the Ba^{2+} concentration of the 238℃ hydrothermal fluid sample reaches 23.2 μ M/kg, which is grouped into the hydrothermal vent sites with high Ba^{2+} concentration. Trace element abundance in oceanic island-arc basalts have been conventionally compared with those of N-type MORB, and the back-arc basalts show relative enrichment in the large low-valency cations K, Rb, Ba and Sr, which are acknowledged as LIL elements transported into the source of island-arc basalt by subduction-zone. Accordingly, it is considered that enrichment of Ba of the vent reflects the characteristics of the hydrothermal system on back-arc basin.

2. Microorganisms in the fluid samples from NKID

No descriptions of deep-sea thermophiles with maximal growth temperature below 80℃ have been reported, which is consistent with the result of FT-IR analysis that showed no archaea existing at the 70℃ vent site. Although the optimal temperature of the archaea observed by the present study is not elucidated before conventional culture study, they inhabit at the comparatively high temperature(70℃) vent sites.

The extremely low content of Ba against high content of S is illustrated in the triangular diagram by the archaea at 238℃ vent site, which is not indicated by the archaea from the 180℃ vent site. It is suggested from this that such S content shows the existence of sulfur(S^0) in the archaea in the sampled fluid at 238℃ vent site. As shown in a pH-Eh diagram for sulfur, the possible state of sulfur species in the hydrothermal environments of the NKID is hydrogen sulfide(H_2S), therefore, the primary electron donors for the archaeal oxidations in the hydrothermal fluids is thought to be hydrogen sulfide(H_2S). In the processes of microbial oxidation of sulfur species, the end product of oxidation is S_4^{2-}, but there can be an accumulation of intermediates, particularly S^0, thiosulfate($S_2O_3^{2-}$), and sulfite(SO_3^{2-}), which is consistent with the EDX result of the archaea from 238℃ vent site.

3. Relationship between saturation state and morphology of barite

The results of past experimental study on barite precipitation indicated that well-formed rectangular, rhombohedral, and polyhedral crystals develop from solutions of low degrees of supersaturation. The same type of barite was found from the inactive chimney fragments, the hydrothermally altered rock, and the suspensions in the fluid sample from the 238℃ vent site.

The concentration product, $(Ba^{2+})i$ ($SO_4^{2-})i$ for the fluid samples from the 180 and 238℃ vent sites with respect to barite are extremely low, and are close to solubility product of each temperature. In such an aqueous state, the morphology of barite crystal should be well-formed crystallization as found from solid samples. However, the barite particles found around the cellular surface of both the bacteria at the 70℃ vent site, and the archaea at the 180 and 238℃ vent site, are quite different in size and morphology.

The crystal morphologies and observation situation are inconsistent with the past experiment and observation. Consequently, it is considered that these barite particles are biogenic rather than abiotic crystallization. That is, the production of such barite particles is cased by biomineralization induced by high-volume production of SO_4^{2-} through the metabolic activities of the archaea observed at the 180, 238 and 311℃ vent sites.

4. Mechanisms of biomineralization of Ba

EDX analyses revealed the archaea possess S^0, which is thought to be accumulated in inclusion bodies within the cell.

The cellular fabric of Gram-negative archaeal has the simplest architecture in comparison with other fabrics, in which S-layers are directly anchored to the plasma membrane like the only cell-wall component of the external to the membrane. This allows the membrane for more dynamic substances transport from the interior to the exterior of the cell.

When sulfate ion is removed outside the cell, it is not thought that the sulfate ion is free in nature, but occurs in combination with organic molecules, which allows the organic molecules to be negatively charged. Dissolved Ba ion around the cellular surface is easily taken in the organic molecules, which causes the formation of crystal nucleation as $BaSO_4$ in the organic molecules. Resultingly, several indeterminate barite particles < 10nm in size are formed, and the organic molecules containing the barite particles develop into the elliptical materials around the archaeal cell.

Unlike the archaea at the 238℃ vent site, S^0 content was not distinguished from the archaea at the 180℃vent site by EDX analysis. This suggests that the archaea at the

180℃ vent site metabolize so actively that none of S^0 is stored within the cell, which results in excreting a large mount of SO_4^{2-} outside the cell to induce supersaturation at high level, and increasing the rate of crystallization of barite. This process is probably caused by their growth temperature. Their growth temperature is presumably higher than that of the archaea observed at the 238℃vent site, and such an environmental stress could determine the archaeal metabolic activity. Sheaths formation resulting in loricate-like outlook is possibly renovated from S-layers, due to comparatively fast reaction time in forming crystal nucleation of barite with the excreted SO_4^{2-} and dissolved Ba ion in the hydrothermal fluid. It is followed from this that S-layered structure is flexible in response to their surrounding environment.

TEM images of the cellular surface show particle distribution of barite, in which the size of the barite particles are not much different, and most popular morphology of the particles in comparatively large size, 10 nm to 17 nm, is elliptic. This morphological feature suggests that these barite particles assemble sequentially in association with an organic molecular, and subunits of new nucleation sites require the barite particles already formed as a template for assembly. Such a sheath formation mechanism can be recognized by the distribution pattern described as "raft foundation".

Analysis of cell morphology focusing on S-layers of various organisms found a strong evidence that Gram-negative archaea possess S-layers as sole cell-wall constituents, and the present study has indicated that biomineralization of barium using the metabolic products owns to their simple cellular fabric, in relation to the surrounding environmental stress such as growth temperature.

CONCLUSION

Biomineralization studies focusing on the deep-sea hydrothermal system in the North Knoll of Iheya Depression, the Middle Okinawa Trough were carried out. Chemical analyses have found the high Ba concentration (23.2 μm/kg in maximum), which reflect a typical characteristics of the hybrothermal fluids on back-arc basin.

The present study has found Gram-negative archaea from the suspensions in the fluid samples from the 180, 238, 311℃ vent sites, and Gram-negative bacteria in the fluid sample from the 70℃ vent site. Additionally, barite particles were observed on the cellular surface, and it has been indicated that their crystal formation processes and conditions are inconsistent with the relationship between the morphology of abiotic barite precipitations and saturation state demonstrated by past experiments.

Biomineralization of barium is closely related to the physiological traits of producing inclusion bodies which serve as a reserve of S^0, and transport system associated the simply structured cell with S-layers. Subsequent crystal nucleation of $BaSO_4$ both inside and outside excreted molecular materials results in forming indeterminate barite

particles 10 nm in size. Furthermore, it has been indicated by the archaea at the 180℃ vent site that S-layers flexible characteristics causes sheaths formation for S-layers, in order to protect the cell from the surrounding environmental stress such as growth temperature. This hypothesis on the archaeal sheaths formation suggests that sheaths formation resulting in loricate-like outlook is possibly renovated from S-layers, due to comparatively fast reaction time in forming crystal nucleation for barite with the excreted SO_4^{2-} and dissolved Ba ion in the hydrothermal fluid. Moreover, it is followed from this that S-layered structure is flexible in response to their surrounding environment.

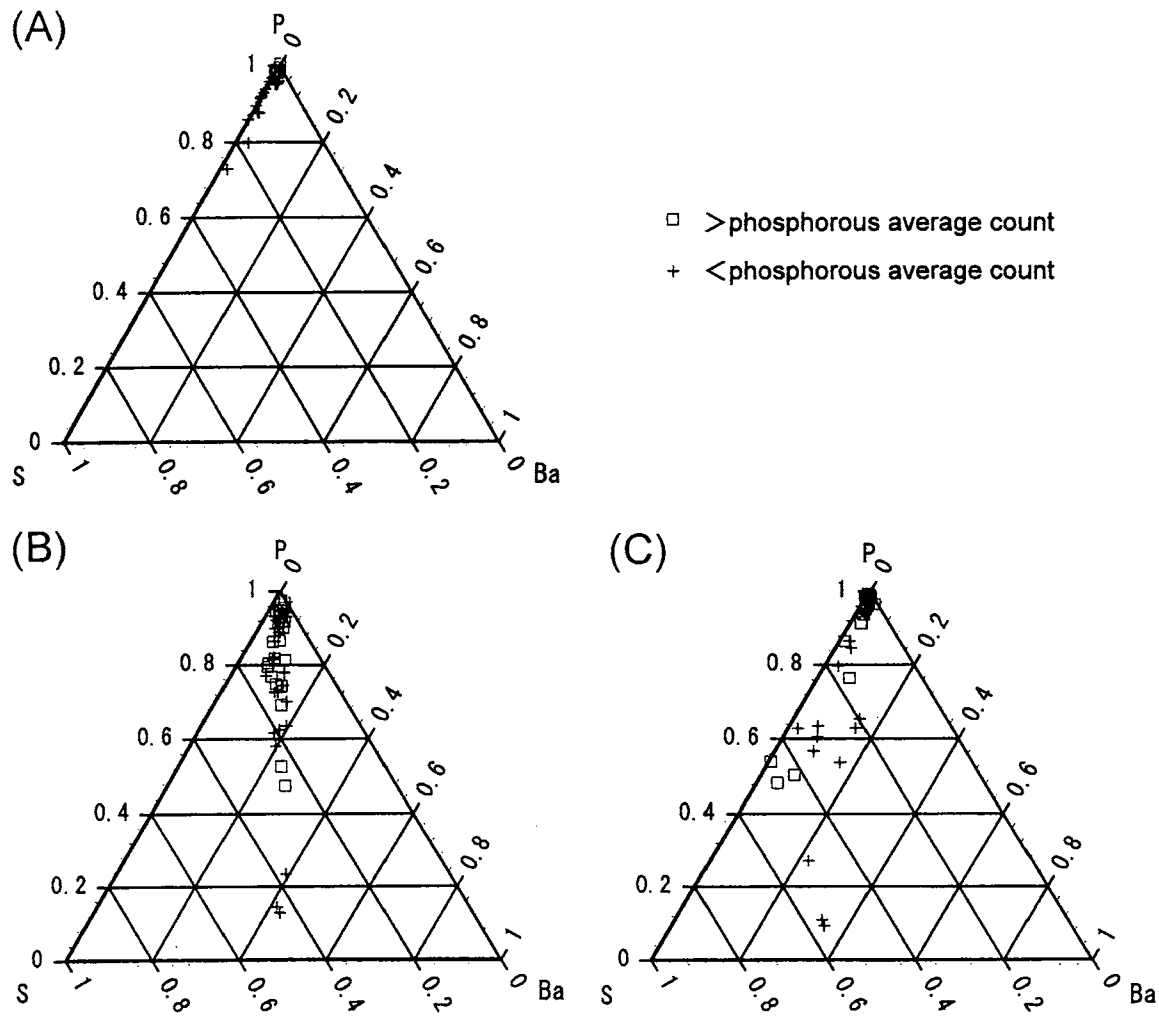

Fig.1 Triangular diagram for P, Ba and S of the microorganisms in the fluid samples at 70 ℃ (A), 180℃ (B) and 238℃ (C) vent site, showing the behaviors of Ba and S in correlation with P. It is noted that all plots are on a straight line from the apex of P to the base in the diagram from the microorganisms at 180℃ vent site.

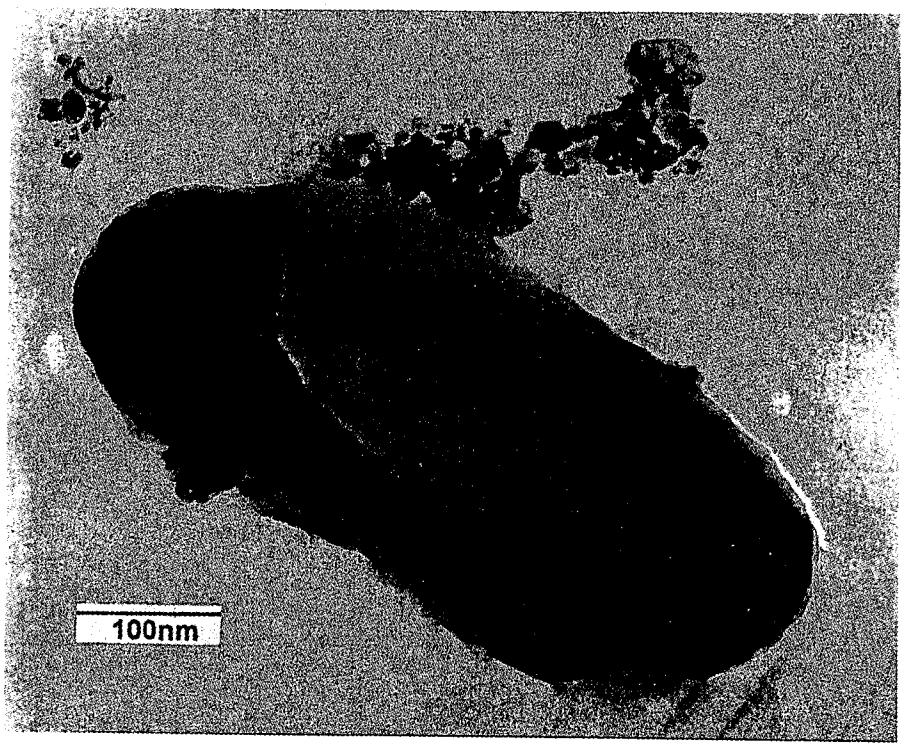

Fig.2 TEM image of Gram-negative archaea from the fluid sample at the 180℃ vent site, showing a formation process of sheath.

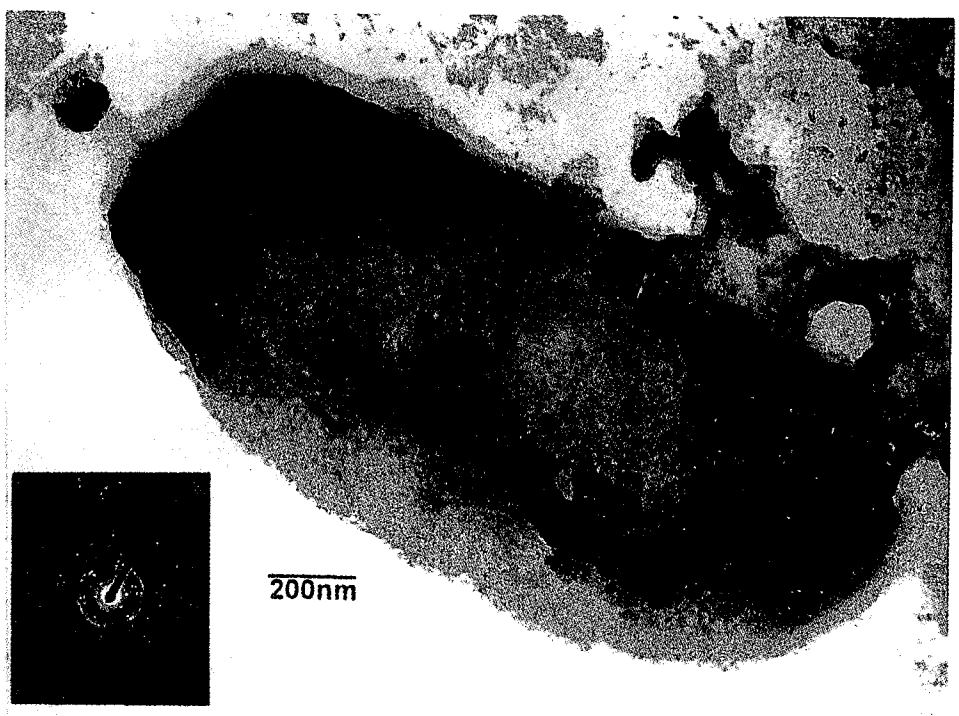

Fig.3 TEM image of Gram-negative archaea in the fluid sample at 238℃ vent site with elliptical materials on the cell, which are characterized as that they are wrapped with filmy organic materials, and involve lesser barite particles inside.

学位論文審査結果の要旨

各審査委員によって，先に提出された学位論文ならびに学位申請書類を審査し，平成12年2月3日に開催した口頭発表を踏まえ，同日審査会を開催し協議した結果，以下の通り判定された。

本論文では，中部沖縄トラフ伊平屋凹地北部海丘の深海底熱水サイトより採取された試料をXRD，EDX-RF，光学顕微鏡，SEM-EDX，EPMAを用いて鉱物学的，化学的に検討し，さらに，FT-IR，SEM-EDX，TEMにより生息する多様な微生物と熱水の化学組成データと比較検討を行いながら微生物，特に古細菌によるバリウムのバイオミネラリゼーションを明らかにした。本論文で得られた成果は次の3点である。

① 本論文では，70，180，238，311℃の熱水噴出孔より採取された熱水試料より微生物を確認したが，FT-IR分析の結果，70℃以上の熱水サイトには，古細菌が生息していることを明らかにした。

② 180，238℃の熱水噴出孔からの熱水サンプル中より観察された古細菌は，バライト粒子をその細胞周辺に集積しているが，これらバライト粒子は，古細菌のバイオミネラリゼーションによって生成されたことを電子顕微鏡をはじめとする各種の分析データで示した。

③ 細胞周辺におけるバライト粒子の生成は古細菌の細胞構造と熱水の化学組成との相互作用によるもので，その生成メカニズムは，生息温度に影響される古細菌の代謝活動によって異なることを示した。

これらの成果は，深海底の熱水環境と生息する微生物活動との関わりを明らかにしたものとして高く評価できる。よって本論文への博士(理学)の授与を認定する。