Quantitative Determination of Fe-oxidation State by Electron Energy Loss Spectroscopy (EELS)

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전자에너지 손실분광 분석법을 이용한 정량적 철산화수 측정

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생지화학적 반응으로 생성된 광물은 화학적 혹은 구조적 변화를 보여주고 있다. 이러한 광물형성의 메커니즘을 밝히기 위해서는 고해상도를 가진 전자현미경을 이용한 나노스케일 분석이 불가피하다. 투과전자현미경에 장착되어있는 전자에너지 손실분광 분석법 (EELS)을 이용하여 미생물-광물반응 시 일어나는 현상을 두 가지의 예를 들어서 설명하고자 한다. 1) 철 환원 박테리아에 의한 노트로나이트의 일라이트로의 전이; 2) 자철석의 환원으로 인한 능철석의 형성. 특히 철산화/환원의 정량적 분석을 통하여 시간적 변화에 따른 철 산화도 측정은 생지화학적 광물변화에 대한 연구를 용이하게 해준다. 따라서 본 논문은 EELS의 분석방법 및 장점을 소개함을 목적으로 한다.

주요어 : 전자에너지 손실분광 분석법, 철산화, 광물전이

The consequences of microbe-mineral interaction often resulted in the chemical, structural modification, or both in the biologically induced mineral. It is inevitable to utilize the high powered resolution of electron microscopy to investigate the mechanism of biogenic mineral transformation at nano-scale. The applications of transmission electron microscopy (TEM) capable of electron energy loss spectroscopy (EELS) to the study of microbe-mineral interaction were demonstrated for two examples: 1) biogenic illite formation associated with structural Fe(III) reduction in nontronite by Fe-reducing bacteria; 2) siderite phase formation induced by microbial Fe(III) reduction in magnetite. In particular, quantification of the changes in Fe-oxidation state at nanoscale is essential to understand the dynamic modification of minerals resulted from microbial Fe reduction. The procedure of EELS acquisition and advantages of EELS techniques were discussed.

Key words : TEM, EELS, Fe-reduction, Mineral transformation

1. Introduction

The characterization of microbially induced mineral formation, particularly nanoscale measurements of structural/chemical changes in mineral structure associated with Fe-reduction give insights of the mechanism of bio-mineralization (Kim *et al.*, 2004; Tazaki and Asada, 2001; Li *et al.*, 2004). The bioreduction depends on type of bacteria that could produce the electron-shuttling compounds (Newman *et al.*, 2000), or chemotaxis (Childers *et*

al., 2002), type of clay that has various sorption capacity (Liu *et al.*, 2001, and surface area (Roden and Zachara, 1996). Previous studies emphasized the extent and rate of bioreduction, and resultant mineral products. However, few attempts have been made to show the dynamic nature of microbial-mediated mineral transformations at the nano-scale. Particularly, few investigation of the changes in elemental Fe oxidation states resulted from microbial Fe-respiration in anaerobic condition was reported. Recently, the spatial variation of the

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oxidation state of chromium when it was reduced by metal reducing bacterium was reported (Daulton *et al.*, 2002). The nanometer scale measurements of the extent of elemental redox states by EELS enables to define the spatial correlation of Cr redox states and microbial activity. Kim and Dong (2011) applied the EELS techniques in the microbially induced mineral transformation study and quantified the extent of Fe(III) reduction in magnetite and siderite. The purpose of present paper is to introduce the EELS application, particularly EELS measurements of Fe-oxidation state to the biomineralization studies.

2. Property of Electron Energy Loss Spectroscopy (EELS)

When the incident high-energy beam interacts with the thin specimen, electrons could scatter elastically or inelastically. Elastically scattered electrons have lost almost nothing from their original energies while inelastically scattered electrons have lost certain amount of their original energies that generate EELS signal. EELS probes, primary events, not secondary (e.g., energy dispersive spectrum (EDS) or Auger electron), are highly localized as such no peripheral fluorescence or interactions occur. EELS signals, thus, result from the direct interactions between the incident beam and the specimen electrons which can measure the energy loss of the inelastically scattered electrons, and can determine specimen electronic structure and oxidation states of transition metal (Leapman *et al.*, 1982; Paterson and Krivanek, 1990; Gracie and Craven, 1994; van Aken *et al.*, 1998; Daulton *et al.*, 2002). Compared with EDS, EELS signal has higher sensitivity to most elements with the energy resolution of 0.3-2 eV giving far fewer overlaps.

3. Acquisition of EELS

EELS measurements were carried out with a JEOL JEM-2010 transmission electron microscope equipped with Gatan imaging filter (GIF200) operating at 200 keV with a LaB₆ filament in the Electron Microscopy Center at Gatan Inc. Pleasanton, California. The following conditions were used during collection of EELS spectra: a convergence angle = 5 mrad, a collection angle = 2 mrad, 2.0-

mm entrance aperture, energy dispersion of 0.6 eV/pixel (0.6 eV/pixel × 1024 pixels = 614.4 eV for total), and energy resolution of 1 eV. Low-loss spectra were acquired with an integration time of 0.1 s (15 spectra summed) and L-edge spectra with an integration time between 0.1 and 1.0 s (between 90 – 200 spectra summed). The diffraction mode of the TEM (i.e., image coupling to the EELS spectrometer) was used to collect EELS signal and followed by correction for dark current and channel-to-channel gain variation of the charge-coupled device (CCD) detector.

Each step for EELS acquisition is described as below:

1) *Location of the interesting area to measure:*
The interested area of sample, for example mixture of example magnetite and siderite associated with the

Fig. 1. TEM micrographs of (a) aggregation of magnetite and siderite at low magnification and (b) the magnified outlined area showing 2.5 and 3.6 Å of layer spacing, respectively. The magnetite and siderite associated with microbial Fe(III)-reduction of magnetite is clearly distinguished in grain size.

microbial Fe(III) reduction was initially investigated with TEM (Fig. 1). Details in the bioreduction experiment of magnetite by *Shewanella putrefaciens* strain CN32 were described in Kim and Dong (2011). Low magnification of TEM micrograph displays aggregates of magnetite nano particles with micron-sized siderite phase (Fig. 1a) and typical 3.6 Å and 2.5 Å spacings for siderite and magnetite were measured (see the magnified outlined area of Fig. 1a) (Fig. 1b).

2) *Measurements of sample thickness by zero-loss peak*: The sample thickness for EELS quantification needs to be suitable in order to obtain EELS data corresponding to single scattering events. For a specimen of thickness t , the average number of times a beam electron will scatter inelastically is t/λ where λ is the mean free path for inelastic scattering. The mean free path, λ , for inelastic scattering represents the average distance a beam electron will travel inside the sample between EELS scattering events. In general, t/λ value of 0.3-0.8 is ideal. The relative sample thickness ($t/\lambda = 0.33$) of the region of interest was measured in EELS signal (Fig. 2) using the log-ratio method ($t/\lambda = -\ln(I_0/I_t)$, I_0 : integrated zero-loss intensity, I_t : the total integrated intensity) using the Gatan's DigitalMicrograph software.

3) *EELS acquisition*: EELS spectrum of zero loss, low loss excitation and core loss ionization

including C-K₁, O-K₁, and Fe-L₂₃ were collected (Fig. 2). The Fe-L edge shows at 700 – 730 eV. The Fe-L edge background was subtracted from L-edge spectra using a standard inverse power law and plural inelastic scattering was removed by Fourier deconvolution methods (red lines). The background-subtracted Fe-L edge for magnetite and siderite showed chemical shift of 1.9 eV (707.3 eV for siderite Fe-L₃ and 709.2eV for magnetite Fe-L₃) and the statistical measurements of the integral ratios of L₃ to L₂ for siderite (2.71) and the bulk magnetite (6.29) were calculated with the Gatan DigitalMicrograph software (Fig. 3).

4. Quantitative Determination of the Extent of Fe(III) Reduction

The valence-induced difference of Fe L₂₃-edges energy loss near edge structure (Taft and Krivanek, 1982) was applied to determine the oxidation state by EELS technique followed by the first findings of changes in valence-dependent white-line intensity ratio of L₃/L₂ adsorption edges (Leapman *et al.*, 1982). Recently, the mechanism of microbial Cr-speciation was reported utilizing a technique of comparing the L₃/L₂ integrated peak ratio of Cr oxidation standards (Daulton *et al.*, 2002). A narrow range of energy-loss by forming images with inelastically scattered electrons can be used to

Fig. 2. The EELS spectrum including the zero-loss (elastic) peak, low-loss region (up to 50 eV), and core-loss region (160 – 800 eV) of the siderite dominant area (Data from Kim and Dong, 2011). The zero-loss peak was used to determine the sample thickness.

Fig. 3. The EELS spectra of magnetite and siderite to determine the Fe oxidation state in each mineral. The background was removed by the standard inverse power law. An energy shift (~ 1.9 eV) of Fe- L_3 edges of magnetite and siderite was measured and the integral ratio of L_3/L_2 for each mineral was calculated (Data from Kim and Dong, 2011).

create the elemental distribution, for example the chemical shift of the Fe L_{23} ionization edges of Fe^{2+} and Fe^{3+} (Golla and Putnis, 2001). As shown in Fig. 3, the integrated background intensity under the Fe- L_2 and Fe- L_3 edges was subtracted from the integrated signal intensity at the Fe- L_2 and Fe- L_3 edge positions. From these measured values the ratio of Fe- L_3 to Fe- L_2 was determined to estimate the Fe oxidation state using the universal curve i.e., the integral ratio of L_3/L_2 versus ferric Fe concentration as determined by van Aken *et al.* (1998) (Fig. 4). The integral ratios of L_3 to L_2 for siderite (2.71) and the bulk magnetite (6.29), calculated by statistic analysis in the DigitalMicograph software, corresponded to $\sim 24\%$, and $\sim 71\%$ of Fe(III), respectively. It should be noted that the range of Fe-oxidation state standards need to be tested in order to determine any unknown with certainty. The universal curve as a function of integral white-line intensity ratio of L_3/L_2 versus ferric iron concentration (van Aken *et al.*, 1998) was modified by Kim and Dong (2011) in Fig. 4. Annite (11% of Fe(III), $L_3/L_2 = 1.92$) from Pikes Peak (Dyar and Burns, 1986), and Muscovite OK-8 (41% of Fe(III), $L_3/L_2 = 3.31$) from Oquossoc

Fig. 4. (a) The EELS spectra of L_3/L_2 -edges of bioreduced nontronite to determine the Fe oxidation state and (b) the universal curve of Ferric iron concentration as a function of integral intensity ratio of L_3/L_2 . The integral ratio of L_3/L_2 was 6.82 for bioreduced nontronite and plotted in the universal curve resulting in 22% of Fe(III) reduction. This result matched with the extent of Fe(III) reduction by 1-10 phenanthroline method.

Fig. 5. The EELS spectra of M_{23} -edges of (a) standard hematite and Lunar ilmenite, and (b) nonreduced nontronite and microbially Fe reduced nontronite. The chemical shift of pre-edge structure between hematite and ilmenite is clearly shown while that of microbially bio-reduced and nonreduced nontronite displays no discernible chemical shift.

Quadrangle, Maine (Dyar, personal communication) were tested, resulting in a good match with the measurements by van Aken (1998). Furthermore, Fe L_{23} -edge of bio-reduced nontronite (NAu-1) was also measured by EELS (Fig. 4a) and integral ratio of Fe L_3/L_2 (6.82) was plotted in the universal curve resulting in 78% of Fe^{3+} (Fig. 4b). This result is compatible with the Fe(II) content measurement by 1-10 phenanthroline method.

Fe M_{23} -edges energy loss near edge structure was also tested using standards (Fe(III) Hematite, and Fe(II) Lunar Ilmenite), showing chemical shift

of pre-edge structure from 52.2 eV for Fe(III) to 54.2 eV for Fe (II) (Fig. 5a). However, plasmon peak may affect the low-loss distribution, for example Fe M_{23} -edges, resulting in the ambiguous pre-edge structures of microbially reduced nontronite (NAu-1) and nonreduced nontronite (NAu-1) in Fig. 5b. The higher energy, Fe L_{23} -edges showed a clear chemical shift.

5. Advantage of EELS

Compared to other techniques measuring elemental oxidation state, such as Mössbauer spectroscopy, EELS has an advantage of measuring oxidation states at a high spatial resolution (the minimum Fe contents of 2-3%). Technically, nanoscale measurement of the interested area of $1\text{ nm} \times 1\text{ nm}$ can be analyzed by TEM with LaB_6 filament. Furthermore, the textural relationship between minerals and microbes is maintained so that the dynamic mechanism of electron transfer between minerals and microbes could be resolved. For example, the EELS technique enables to trace the changes in Fe redox state at different stages of magnetosome formation (intracellular magnetite crystals inside magnetotactic bacteria). One condition for quantifying Fe(II) and Fe(III) is that the energy resolution of the EELS instrument has to be higher than the chemical shift of the L_{23} edges. The extracted EELS signals with 1.9 eV chemical shift for Fe-oxidation state in this study would be enough to identify the Fe-redox state using the energy resolution of EELS of 1 eV.

Acknowledgement

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (20110013407) to Jin-wook Kim,

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2012년 3월 29일 원고접수, 2012년 4월 20일 게재승인