Observation of Biofilms by SR Infrared Microscopy

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Abstract

Infrared (IR) spectra of biofilms were observed by synchrotron radiation IR microscopy. Typical spectra observed in biology cells were obtained in several samples. However, different NH vibrations were confirmed in the spectra of one of the samples although all types of vibration components in the spectra were the same as the others. This suggests that the states of NH groups were different from those of typical samples. Analyses of these spectra can be necessary for investigation of the structures of the biofilm in detail.

1. Introduction

Biofilms are aggregates of microorganism forming community structure and live in aquatic environments such as algae in a lake and water pipes. Studies of their formation processes and structures can be therefore useful for improvement in our living environment. The growing processes of the biofilms have been studied using the way in surface science and physical chemistry [1]. However, information on chemical composition and their interactions in biofilms can be also important to clarify them. Infrared (IR) microscope can measure IR spectra in microscopic region and is a useful tool for investigation of molecular components and their states in a biofilm.

In this paper, we observed IR spectra of biofilms and investigated characteristics of vibrational components of biofilms.

2. Experimental Section

Biofilms were grown on glass substrates set in an artificial pond in Ritsumeikan University and sampled from the substrates after a month. The samples were prepared by putting a droplet of the medium on ZnSe substrates, and their IR spectra were observed using synchrotron radiation IR (SR-IR) microspectroscope beamline (BL-15) in the SR center. The IR microscope is ContinuμmXL connected with Nicolet 6700 FT-IR spectrometer (Thermo Scientific Inc.). Spectra were collected over mid-infrared range 4000-800 cm^{-1} using a 20 μm square aperture in transmittance mode.

3. Results and Discussion

One example of an observed biofilm (sample A) is shown in Figs. 1. The biofilm A included some algae. The IR spectra of the biofilm A are shown in Fig. 2. All spectra in this text are averaged over the center region of the algae and did not include those of the edge region because the spectra of the edge were affected by scattering due to shape and size effects of the samples and resulted in wavy baselines. Assignments of the peaks in the averaged spectra to vibration components are as follows [2]: In the higher wavenumber region (Fig. 2(a)), a broad absorption band over 3700-3000 cm^{-1} was assigned to O-H stretching vibration (ν(OH)), a sharp peak at 3344 cm^{-1} on ν(OH) was to N-H stretching vibration (ν(NH)) which is observed in amino acids and proteins (in case of proteins is called Amide-A band), and the four peaks in 3000-2800 cm^{-1} were to CH_3 anti-symmetric stretching vibration ($\nu_{as}(CH_3)$, 2963 cm^{-1}), CH_2 anti-symmetric stretching ($\nu_{as}(CH_2)$, 2923 cm^{-1}), CH_3 symmetric stretching ($\nu_s(CH_2)$, 2872 cm^{-1}), CH_2

symmetric stretching ($\nu_s(CH_2)$, 2852 cm^{-1}). In the lower wavenumber region (Fig. 2(b)), a small peak at 1740 cm^{-1} was assigned to C=O stretching vibration of ester group (ν(C=O)), next two broad peaks at 1650, 1550 cm^{-1} were to (ν(C=O)) stretching and N-H deformation (δ(NH)) of Amide group, called Amide I, Amide II, respectively. A broad band at approximately 1430 cm-1 could be produced by the superposition of deformation vibrations of OH, CH_2 and CH_3 (δ(OH)+ $\delta(CH_2)$ + $\delta(CH_3)$), not only one vibration component. Continuous peaks in 1300 cm^{-1} to 1200 cm^{-1} could be Amide III band, which is composed of many vibrational modes of amide bonds in protein. Some peaks in a region of 1200 cm^{-1} to 1000 cm^{-1} can be assigned to C-O stretching vibration and ring vibrational modes, frequently observed in saccharides. Complex spectra composed of many vibrational modes were observed in the low wavenumber region as described above, which is called 'finger print' region and has characteristic structures of each molecule. However, some peaks of functional groups are in the region. This sample has two bands at 1232 cm^{-1} and 1060 cm^{-1}, assigned to anti-symmetric stretching vibration ($\nu_{as}(P=O)$) and symmetric stretching vibration ($\nu_s(P=O)$) of P=O in nucleic acids. The samples give the same vibrational

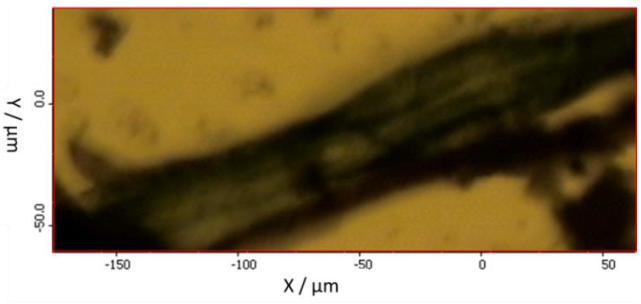

Fig. 1 A topological image of a biofilm sample A. A large object is mainly algae.

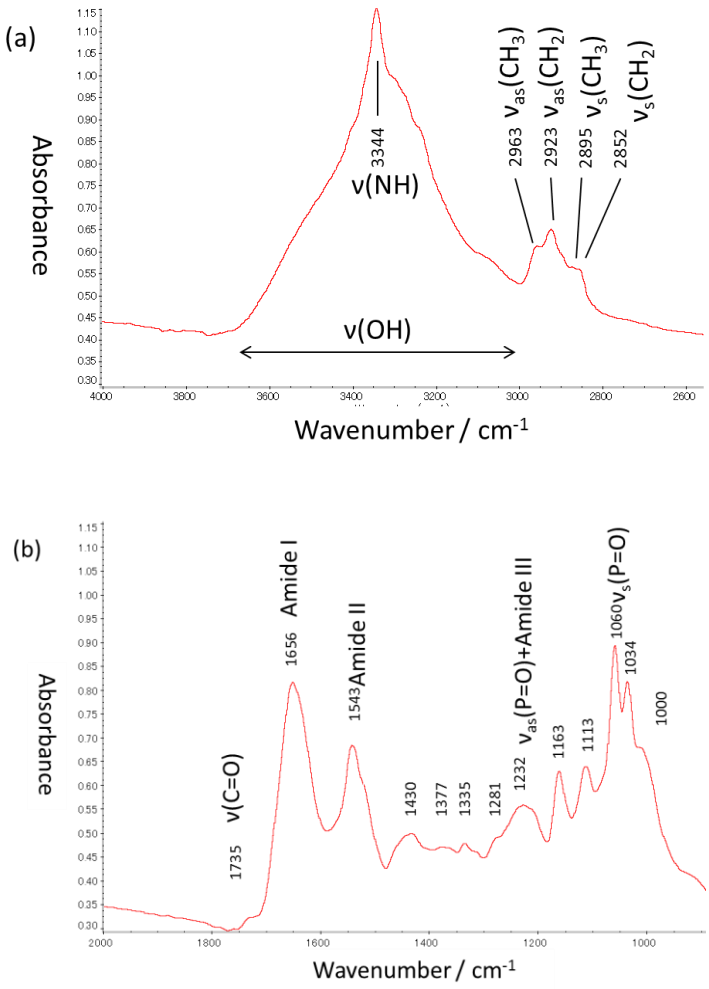

Fig. 2 Averaged spectra of sample A, obtained in the higher wavenumber region (a) and the lower wavenumber region (b), over the center region of the algae in Fig. 1.

components, typically observed in biological cells composed of saccharides hydrocarbon, lipids, protein, and nucleic acids. Several samples were observed and the same spectra were obtained. We have recognized the observed spectra are typical for the present samples.

Another sample B has almost the same size as sample A (Fig. 3), but has a different structure in the observed spectra from the sample A as described below. The averaged spectra of the sample are shown in Fig. 4. Assignments of the spectra were almost the same as those of sample A. In the higher wavenumber region (Fig. 4(a)), the band of 3700-3000cm-1 was assigned to ν(OH), two bands at 3445 and 3287 cm^{-1} to ν(NH), and four bands in 3000-2800 cm^{-1} to $\nu_{as}(CH_3)$ at 2960 cm^{-1}, $\nu_{as}(CH_2)$ at 2920 cm^{-1}, $\nu_s(CH_2)$ at 2874 cm^{-1} and $\nu_s(CH_2)$ at 2849 cm^{-1}, respectively. In lower wavenumber region (Fig. 4(b)), ν(C=O) in ester group at 1735 cm^{-1}, Amide I at 1652 cm^{-1}, Amide II at 1539 cm^{-1}, δ(OH) + $\delta(CH_2)$ + $\delta(CH_3)$ in a region of 1480-1280 cm^{-1}, Amide III around 1200 cm^{-1}, $\nu_{as}(P=O)$ at 1239 cm^{-1} and $\nu_s(P=O)$ at 1000 cm^{-1} were observed. and the same vibrational components as in sample A were obtained. However, some vibrational structures are clearly different from those of sample A. They are NH

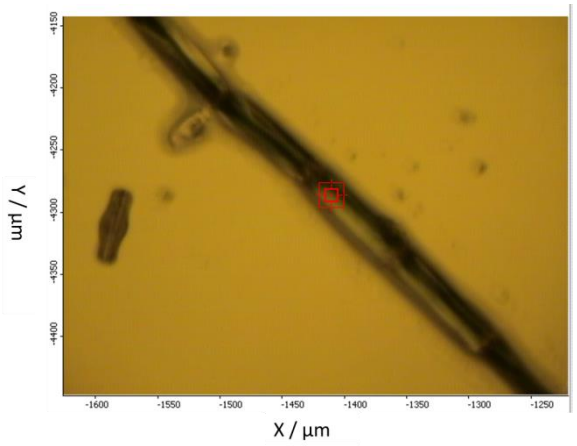

Fig. 3 A topological image of another biofilm sample B. A dark object is mainly algae.

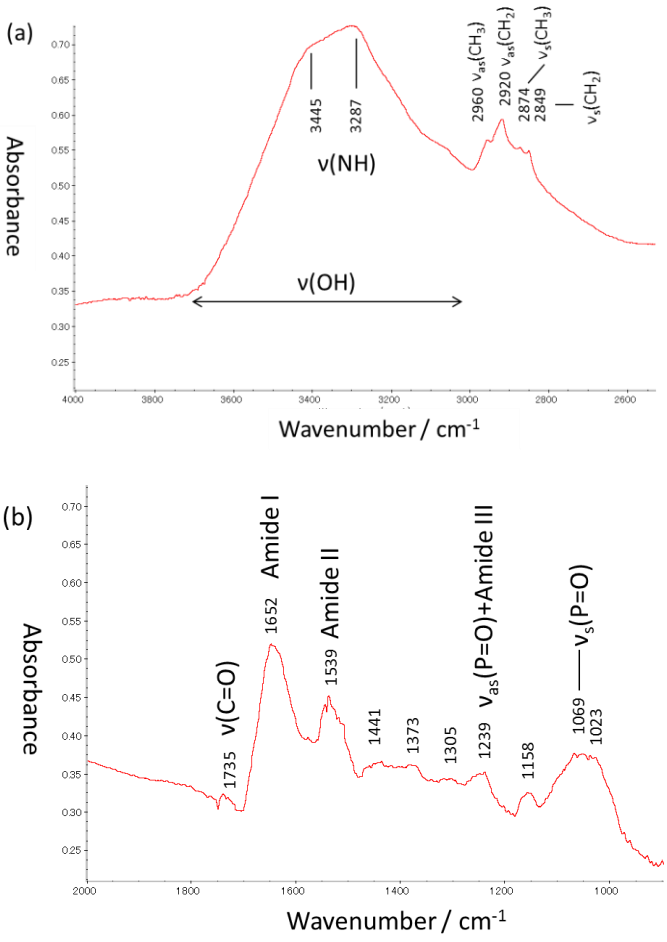

Fig. 4 Averaged spectra of sample B, obtained in the higher wavenumber region (a) and the lower wavenumber region (b), over the center region of the algae in Fig. 3.

stretching vibrations. One sharp peak is at 3344 cm^{-1} in sample A while two small split bands are observed at 3445 cm^{-1} and 3287 cm^{-1} in sample B. In general, splitting and shift of IR absorption bands can occur due to a change of states of functional group caused by intra- and/or inter-molecular interaction. This suggests that molecular structure around NH groups in samples B were different from the other samples. We cannot conclusively discuss interactions between biofilm and algae or between biofilms because the average spectra taken over the center of the algae can mainly reflect information of the structure in algae. If the averaged spectra in sample B are similar to the spectra near edge of the sample, the spectra are expected to have information on an interface between biofilms and algae. In order to discuss these results in detail, we must also analyze their spectra and compare with the averaged spectra, as well as additional observations of the biofilm samples. The wave-like baselines of their spectra are necessary to be corrected by statistical techniques [3]. These are our next subjects.

4. Conclusions

We observed IR spectra of biofilms by SR-IR microscopy and obtained typical spectra of biofilm sample. However, the NH vibration split into two band was confirmed in one of the samples although all types of vibration components in the spectra were the same as the others. The result suggests that the states of NH groups were different from those of typical spectra. The biofilm comparison of their spectra will be essential. We plan to compare their spectra with the spectra near the edge including information on interface of the biofilms.

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