A Dimeric Structure of Bacteriochlorophyllide *c* Molecules Studied by Scanning Tunneling Microscopy

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Scanning tunneling microscopy (STM) has been employed to study the organization of photosynthetic bacteria. A high-resolution spatial image is acquired on a bacteriochlorophyllide *c* (BChlide *c*) monolayer prepared by a simple method. BChlide *c* molecules are found to self-organize a well-defined adlayer on a highly oriented pyrolytic graphite (HOPG) surface. A dimeric structure different from those observed in Chls *a*, and *c* and BChlide *d* is first revealed in the adlayer. A hydrogen bond is assumed to exist in the dimer between the OH groups at C_3^1 positions.

Introduction

Bacteriochlorophyllide *c* (Bchlide *c*), shown in Figure 1, is a derivative of Bacteriochlorophyll *c* (BChl *c*), which exists in green photosynthetic bacteria chlorosomes as a major light-harvesting pigment. The association structure of both bacterio-chlorophyllide and chlorophyll is one of the most highly focused subjects in the study of photosynthetic membranes. A considerable body of spectroscopic data such as IR and NMR (nuclear magnetic resonance) has provided average information for dimerization, aggregation, and chlorophyll−protein interactions to account for the various BChl forms observed in vivo.[1−10] For example, a 70 nm red shift from the monomeric form of BChl *c* was evidenced by an absorption maximum (Q_y) at 740 nm within the near-infrared region in spectroscopic measurements.[1,2] An intermediate form of intact BChl *c* in carbon tetrachloride (CCl_4) was observed by NMR.[9,10] The results showed that BChl *c* forms highly stable dimers characterized by an asymmetric antiparallel "piggy-back" conformation and a Q_y absorbance at 710 nm. However, the information obtained by these techniques is rather macroscopic and does not provide direct atomic-scale models. On the other hand, new in situ techniques such as scanning tunneling microscopy (STM) have been well-recognized as important methods for structural investigations of various organic molecules on well-defined electrode surfaces with atomic resolution. Examples include adsorbed TMPyP, crystal violet, and aromatic molecules on Au, Ag, Cu, highly oriented pyrolytic graphite (HOPG), and other surfaces.[11−13]

In this letter, we, for the first time, report an in situ STM result of the molecular arrangement of BChlide *c* on a HOPG surface. BChlide *c* molecules are found to self-organize a well-defined adlayer on the HOPG surface. Association of the molecules in the adlayer results in the formation of a dimer structure, which is assumed to form by hydrogen-bond interaction between
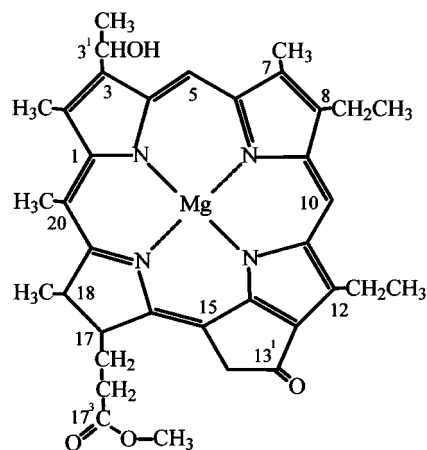
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Figure 1. Chemical structure of BChlide *c* molecule.

the OH groups at the C_3^1 position. The dimeric structure is different from that of Chls *a* and *c* and BChlide *d* reported.[3−10,14−17] Based on the STM observations and theoretical calculations, a structural model is proposed for the molecular arrangement.

Experimental Section

BChlide *c* pigments were prepared from *Chlorobium limicola* cells by using the method described by Chow et al.[18] The extracted BChlide *c* mixture was further purified by a reverse-phase HPLC system as reported previously.[19] The most abundant component, 8-ethyl-12-ethyl-[*E*,*E*]-BChlide *c* was used for the STM experiment. BChlide *c* molecules were dissolved in methanol. A droplet of the solution was deposited onto a freshly cleaved HOPG surface. After the methanol evaporated, a BChlide *c* film was formed on the HOPG surface. STM experiments were performed with a Nanoscope III SPM (Digital Instruments) in ambient conditions at room temperature between 22 and 25 °C. A Pt/Ir tip was used. The details for STM experiment are the same as those described in the literature.[12,13] The molecular model was constructed by Insight II software in a SGI workstation.
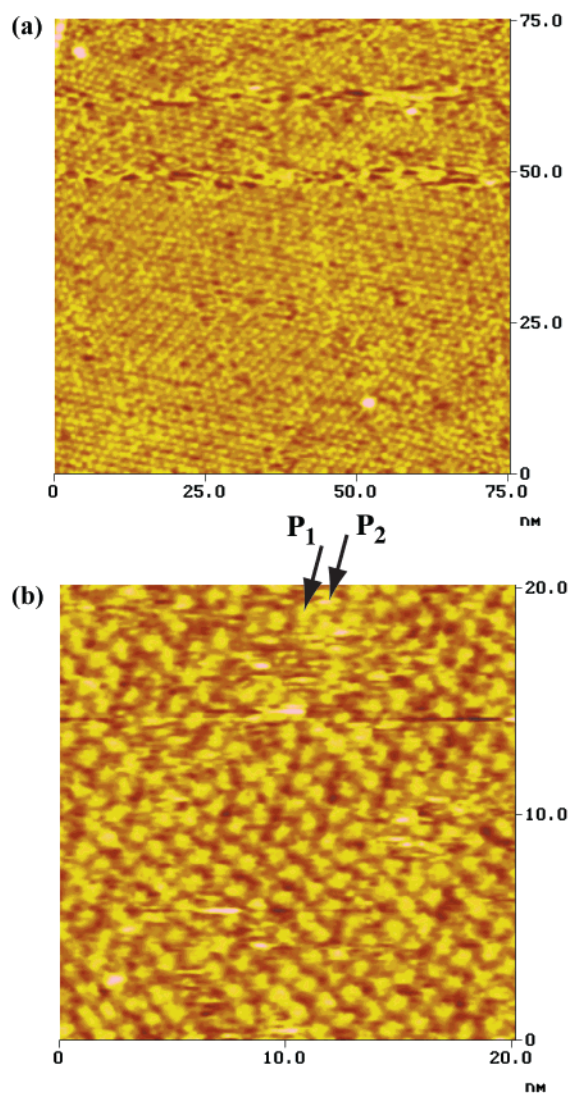
Figure 2. Large-scale STM images of the BChlide *c* molecular adlayer on the HOPG surface. The tunneling current was 730 pA; the sample bias was 740 mV.

Results and Discussion

A Langmuir-Blodgett (LB) method was used in previous research, but the image resolution of the film obtained by STM and AFM was not good enough to give reliable information.[16,17] Because BChlide *c* molecules cannot be imaged in vivo by STM, they have to be fixed on a solid substrate. In the present study, the molecules were dissolved in methanol. A monolayer of BChlide *c* was formed on the HOPG surface. STM could be used to acquire stable and high-resolution images on the adlayer. It was found in STM imaging that the molecules form a self-assembled monolayer. An individual BChlide *c* molecule was well resolved. Association of the molecules in the monolayer results in the formation of a dimer structure different from that of Chls *a* and *c* and BChlide *d* reported.[3-10,14-17]

Figure 2a,b shows typical STM images of the BChlide *c* molecular adlayer. It can be clearly seen in the 75 nm × 75 nm area of Figure 2a that BChlide *c* molecules uniformly adsorb on the HOPG surface and self-organize a well-ordered monolayer. Several domains with the same atomic feature rotate by 60° or 120° from each other within the experimental error of ±2°. Although molecular defects were sometimes observed in the monolayer, especially in the domain boundary, the two-dimensional network can be reproducibly observed over a large

area, indicating a high stability of this self-assembly. The higher-resolution STM image reveals the adlayer details. Figure 3a,b shows a top view and a height-shaded surface plot, respectively. A low-pass filtered transform was employed to remove scanning noise in the STM images. It can be seen from Figure 3a that the molecular layer consists of ordered bright spots with an approximate size of 1 nm. Each spot is well-resolved and assumed to correspond to a BChlide *c* molecule. A depression can be seen in the center of each bright spot from the height-shaded surface plot (Figure 3b). Considering the chemical structure of BChlide *c*, this depression should be the center of a BChlide *c* macrocycle. The average corrugation height of the depression is ca. 0.01 nm. According to the ordering symmetry of the two-dimensional molecular adlayer and intermolecular distance, a unit cell is outlined in Figure 3a. Dimensions of the unit cell are measured to be $a = 2.5 \pm 0.2$ nm and $b = 1.2 \pm 0.2$ nm along the A and B directions, respectively. The angle between the two directions is $68° \pm 2°$. On the other hand, a pairlike appearance can be clearly seen in the STM images of Figures 2 and 3. All molecular rows appear in a pairing structure composed of row p_1 and p_2, as indicated in Figure 2b. Two molecules in a pairing row constitute a dimer, which is a basic building element for the adlayer. The center-to-center distance within a dimer is approximately 1.0 ± 0.2 nm. The dimeric structure is reproducible and very stable. Varying imaging conditions such as scanning rate, direction, and angle did not change the structural feature.

The dimeric feature was previously reported in Chls *a* and *c* and BChlide *d* in an LB film or in vivo.[3-10,14-17] Various models for the dimeric structure were proposed for these molecules. For example, atomic force microscopy (AFM) and STM were used to image an LB film of Chl *a*.[16,17] The obtained images were interpreted by a dimeric model proposed for the primary electron donor of photosystem I. Two water molecules are involved in linking the two Chl *a* molecules, that is, the oxygen of a water molecule is coordinated to the magnesium of a Chl *a* molecule, while its hydrogen atom bonds to the keto carbonyl group of a second Chl *a* molecule. The Chl *a* macrocycles are probably tilted to each other by 30° with respect to the membrane plane. In the cases of BChl *c* aggregates, the 3^1-hydroxyl oxygen is supposed to directly coordinate to the Mg atom of a second molecule.[9,10] Unlike Chl *a*, a small amount of water does not seem to markedly affect the properties of BChl *c* and BChlide *c* aggregates. This is probably due to the fact that the BChl *c* has a nucleophilic group (3^1-hydroxyl) as a part of the molecule. In the BChlide *d* dimer, Mg atoms are coordinated to the C-2 hydroxyl of the adjacent molecules, and the separation between the parallel macrocyclic planes is ca. 0.35 nm, with Mg–O distances of ca. 0.26 nm.[14,15] However, most of these models were deduced from indirect spatial and average information in vivo. To our best knowledge, no spatial and atomic resolution result for BChlide *c* dimer was reported even in ambient on a solid film. Therefore, first, we try to get high-resolution STM images such as Figures 2 and 3, and then theoretical calculations were carried out to construct the structural model of BChlide *c* on HOPG.

In general, molecules are physically adsorbed on the HOPG surface.[12,20] In the present study, the formation of the well-defined molecular adlayer consisting of dimeric structure is considered to mainly rely on the interaction among molecules through hydrogen bonds. From the chemical structure of the BChlide *c* molecule, there might be three possibilities to form hydrogen bonds in a dimer: (1) between the hydroxyl groups at C_3^1 position, (2) between the hydroxyl group at C_3^1 and the

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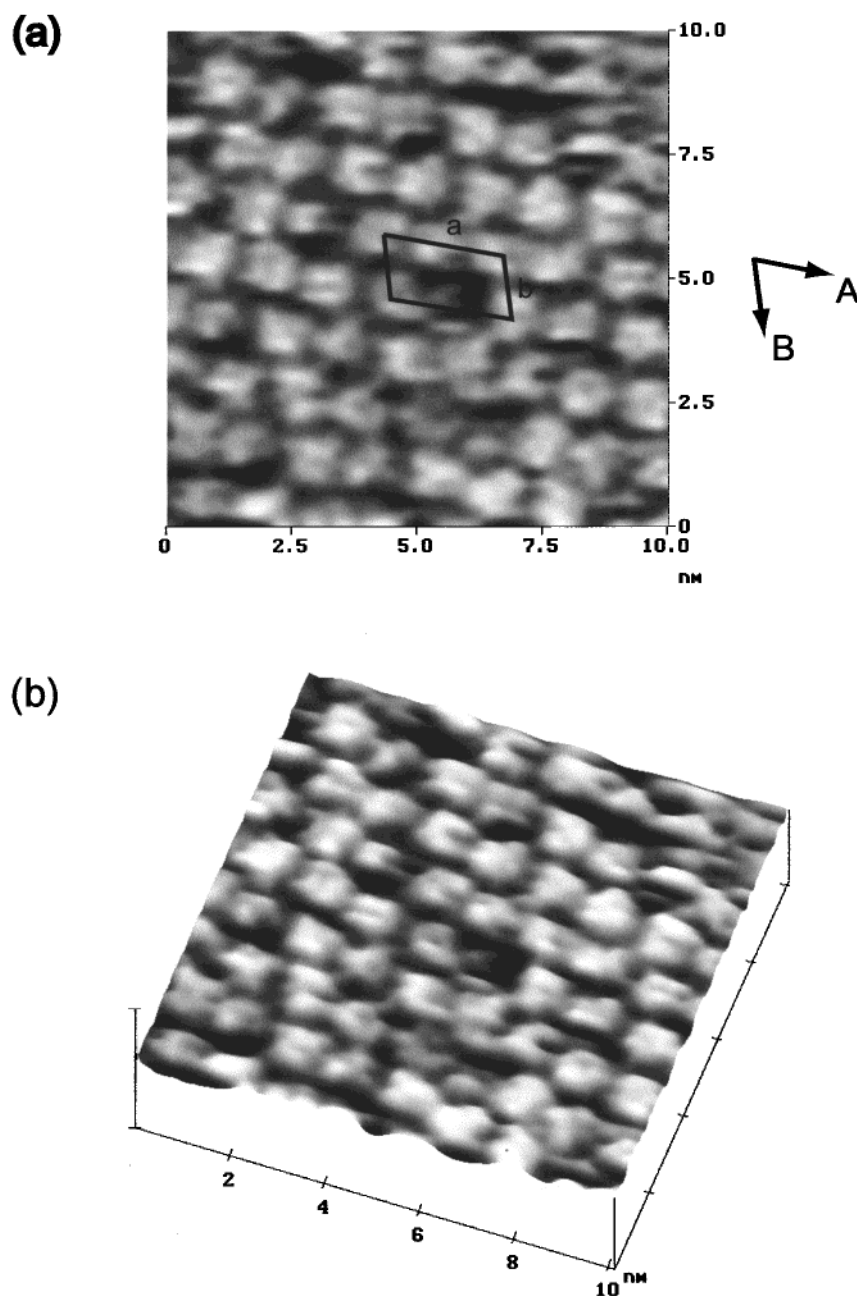
(a)

(b)

Figure 3. Top view (a) and height-shaded surface plot (b) of BChlide *c* molecules. Imaging condition was 400 pA and 800 mV.

carbonyl group at $C_{13}{}^1$ position, and (3) between the hydroxyl group at $C_3{}^1$ and the carbonyl group at $C_{17}{}^3$ position. The potential energy for a BChlide *c* dimer with the above three possible hydrogen-bond configurations was calculated by using the molecular mechanics method and the semiempirical quantum mechanics ZINDO/1 method by Discover package. The results show that the potential energy in the first configuration is lower than that in the latter two configurations by 12.51 and 91.65 kcal/mol, respectively. That is to say, the dimeric structures probably stem from the hydrogen bonds between OH groups at $C_3{}^1$ position of BChlide *c* molecules. On the basis of STM results and theoretical calculations, we propose a structural model for the BChlide *c* adlayer shown in Figure 4. The parameters of the unit cell deduced from Figure 4 are $a = 2.7$ nm, $b = 1.4$ nm, and the angle between a and b is 70°. The center-to-center distance in a dimer is 1.2 nm. The parameters are in good agreement with the observed STM images. In this model, the BChlide *c* molecules are almost lying flatly on the

HOPG surface, that is, the molecular planes are almost parallel to the HOPG substrate. The molecular rows p_1 and p_2 constitute a pairing structure as that in the STM images of Figures 2 and 3. The neighboring two molecules along the A direction in a pair form a dimer through hydrogen bonds between hydroxyls of $C_3{}^1$. A side view of the model shows the hydrogen bond positions. Oxygen atoms in hydroxyls of $C_3{}^1$ are coordinated with hydrogen atoms in hydroxyls of $C_3{}^1$. The dimer configuration in the present study is different from those for Chls *a* and *c* and BChlide *d* in an LB film or in vivo.[3−10,14−17] The chemical structure and the sample preparation for the adlayer are considered to be responsible to the difference. The new dimer structure in Figure 4 should be important to the study of the organization of photosynthetic bacteria.

Conclusion

A simple method has been used to prepare monolayer BChlide *c* adlayer for structural investigation. BChlide *c*
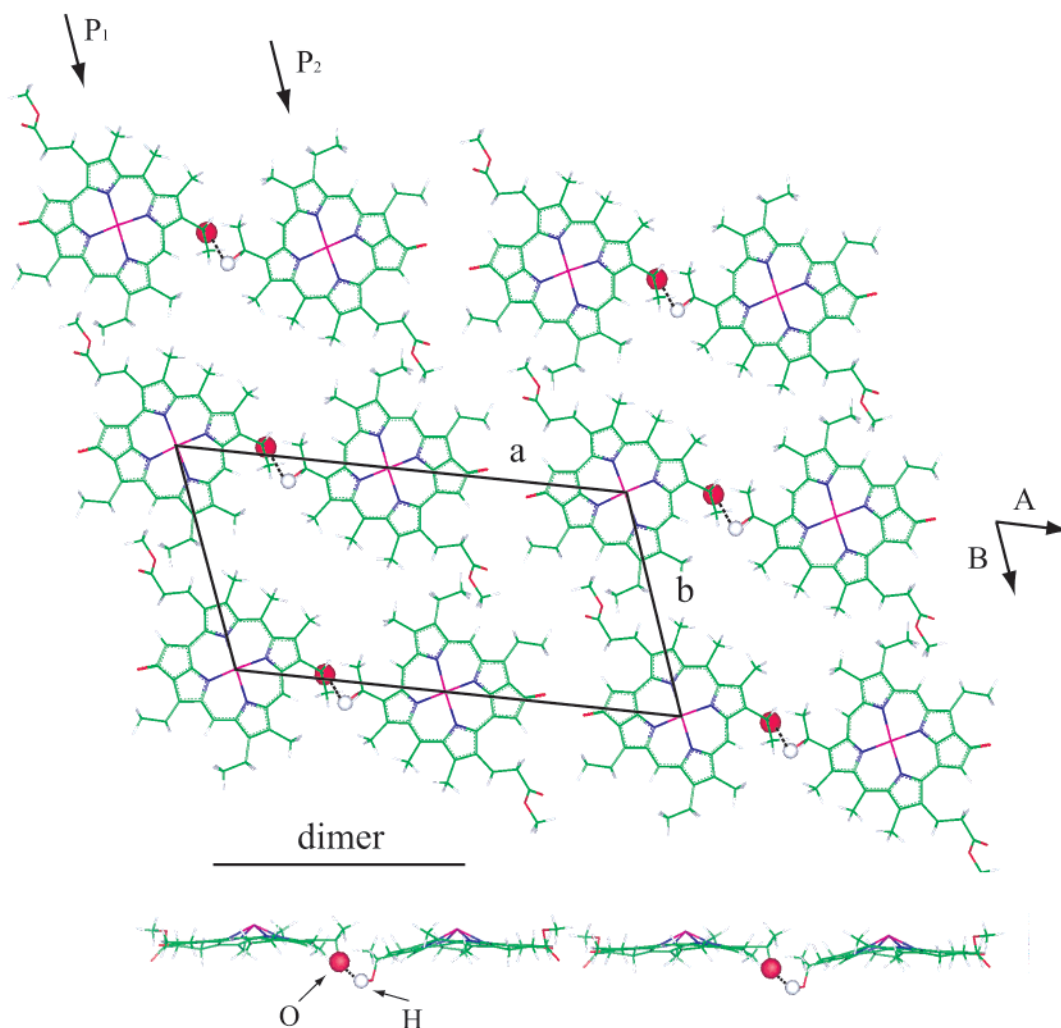
Figure 4. A top view and side view of a proposed model for the BChlide *c* molecular adlayer. The filled red circles correspond to oxygen atoms and the open blue circles to hydrogen atoms. The hydrogen bond is formed between the two atoms.

molecules are found to self-organize a well-defined adlayer on a HOPG surface. In situ STM discloses a dimer structure in the BChlide *c* adlayer. The dimer is different from those observed in Chls *a* and *c* and BChlide *d* in an LB film or in vivo. A hydrogen bond is assumed to exist in a dimer between the OH groups at the C_3^1 position of two BChlide *c* molecules. A model from STM observation and theoretical calculation is proposed to explain the dimeric structure. The success in this study should shed new insight into the investigation of photosynthetic bacteria.

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