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Scanning tunnelling microscopy of a monolayer of reaction centres

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Abstract

Langmuir–Blodgett monolayers of photosynthetic reaction centres from *Rhodobacter sphaeroides* have been studied by scanning tunnelling microscopy. Freshly deposited films were studied both in the dark and in the light. In the dark, images revealed molecular structure with 64 Å and 30 Å periodicities, which correspond to protein and sub-unit sizes known from X-ray crystallography, while no periodic structure appeared in the light due to the tip action on the excited proteins. STM voltage–current measurements showed the charge separation in single protein molecules in the film and their different behaviour in the dark and light. Together with surface potential measurements at the macroscopic level, they indicated the preservation of reaction centre activity in the monolayer. By fixing the protein layer with glutaraldehyde, it was possible to prevent the perturbing tip action and obtain a periodic molecular structure with 30 Å spacing even in the light. After heating at 150 °C, the unfixed film reorganized itself into a long-range ordered state with a hexagonal structure of 27 Å spacing but with no activity.

1. Introduction

Scanning tunnelling microscopy (STM) [1–4], together with atomic force microscopy (AFM) [3, 5, 6], is considered as one of the best techniques for imaging large organic molecules. In addition, it can display its future potential even better when applied to the investigation of molecules, such as reaction centres (RC), with functional properties detectable by electrical measurements. In this case, STM can give information about the structure and the functionality of the sample. In addition, measurements of structural and functional parameters of a single RC molecule can be achieved only by means of nanoprobes, sensitive to current, capable of very high resolution (such as an STM tip).

Reaction centres from *Rhodobacter sphaeroides*, a large (100 kDa) protein formed by 3 sub-units [7] and carrying out light-induced electron transport across bacterial membranes [8], have been the subject of detailed investigations [9, 10] because they enter the photosynthesis process and are among the most promising molecules for bioelectronics. The existence of electron transfer chains inside this protein facilitates the flow of current through it and indicates that RC is one of the best subjects for STM study among proteins.

In order to exploit the properties of RC as active elements of bioelectronic devices, it is useful to organize them in a regular two-dimensional lattice [11] and to study their structure and function, and the ways in which these properties depend upon temperature. The Langmuir–Blodgett (LB) technique provides these regular structures and is considered to be one of the most useful technologies for bioelectronic purposes [12].

Here, we report an STM study of structural and functional properties of LB RC monolayers deposited on highly oriented pyrolithic graphite (HOPG) and imaged in air.

2. Experimental details

RC (Moscow State University) [13] were arranged into a film with an LB trough (MM-MDT Co.) according to the standard procedure [14] and transferred onto HOPG substrate at 35 mN m^{-1} surface pressure by the horizontal lift technique [15]. Purified (Milli-Q) water with resistivity of 18 MΩ cm was used as the subphase. The HOPG substrate was found to be suitable for protein STM imaging [3].

For STM measurements we used a device (MM-MDT Co.) suitable also for voltage–current (V–I) characteristic measurements at a single point. All the images were obtained in air with the same tip (PtIr 80:20) in constant current mode (after obtaining atomic resolution on HOPG) within a tunnelling current range of 0.1–0.5 nA and voltage from −1.5 V to 1.5 V. Outside the specified voltage range it was impossible to obtain acceptable noise values and meaningful images. By increasing the current up to 1 nA, the graphite lattice under the protein layer appeared, allowing calibration of the images.

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For $V-I$ characteristics, after obtaining each image in the dark the tip was moved to several detectable bumps with feedback switched on; then a weak current (5 pA) was locked, in order to be sure to be out of the film. Then the voltage was swept in the range -4–4 V and the tunnelling current was recorded. In the case of irradiation, as no corrugation was detectable the position of the tip was chosen randomly over many different points of the scanned areas. In all cases, however, the results were almost identical to that here reported.

Surface potential measurements were performed on deposited monolayers by means of a Kelvin probe (vibrating electrode) [16].

3. Results and discussion

The film prepared according to the procedure described above was first studied without modifications. It was imaged in the dark (Fig. 1) and in the light. Irradiation was provided by a 100 W tungsten lamp, using a 3 cm water filter [9]. In the dark, the images revealed two different periodic structures: a low-frequency one, giving average spacing value of about 64 Å and a higher frequency one corresponding to a periodicity of 30 ± 1 Å. In the light, no periodic features but graphite steps were obtained (data not shown). We think that the higher frequency corrugation can account for the sub-units constituting each molecule [17], while the 64 Å periodicity correlates well with dimensions of the protein molecule. These values are in agreement with the protein size induced from X-ray crystallography data of RC structure from the Protein Data Bank [18].

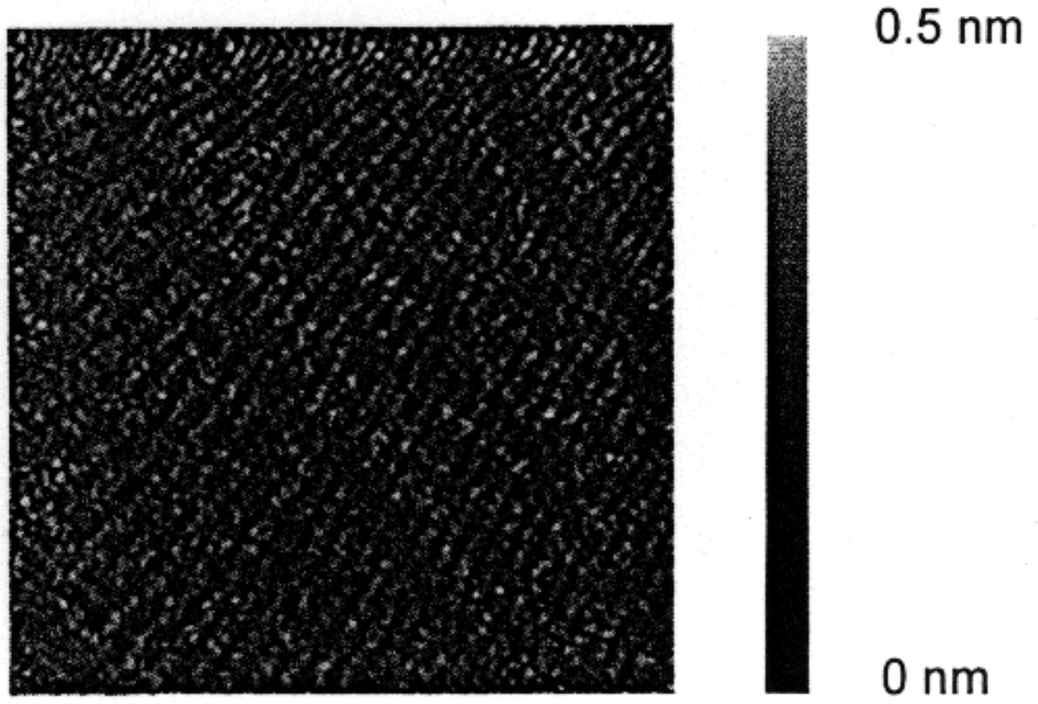


Fig. 1. STM image of an RC monolayer in the dark. Image size is 57.6×57.6 nm^2. Constant current mode image. Tunnelling current was set at 0.15 nA and tunnelling voltage was 1 V (tip positive); the scanning frequency was 2 Hz. The effect of the tilted substrate was compensated by software.

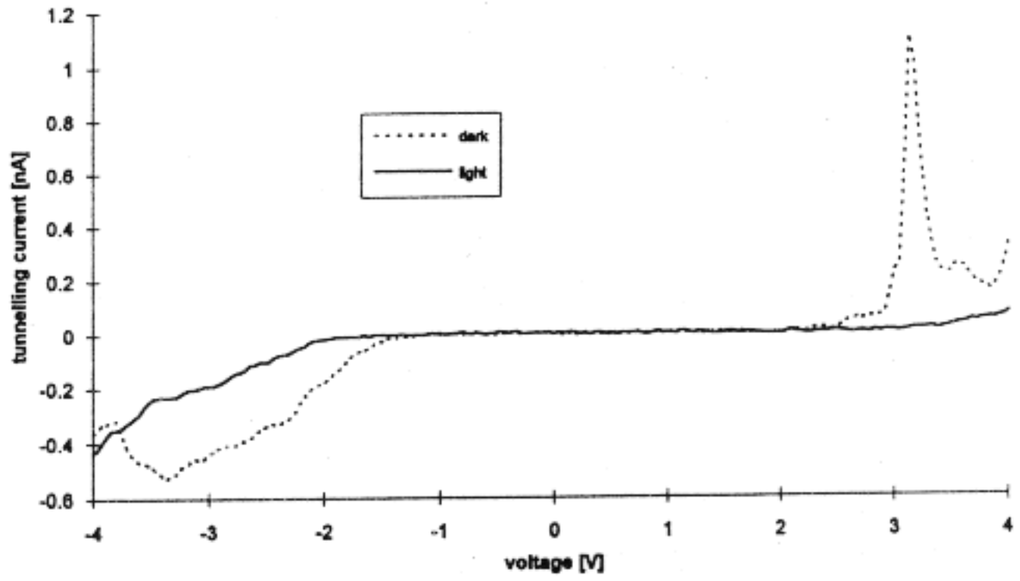


Fig. 2. Voltage–current characteristics of HOPG–RC–STM tip system in the dark (dashed line) and in the light (solid line).

$V-I$ characteristics at single points were measured both in the dark and in the light to try to show the functional activity of the single RC protein (Fig. 2). The results outline the basic difference in the RC film under two different experimental conditions. In fact, the $V-I$ characteristics measured in the dark on several bumps show a resonance at a tip–substrate voltage of 3.16 V. This peak disappears completely in the light. The resonance should be due to the functional properties of RC, namely the electron transfer process between the bacteriochlorophyll donor and the quinone acceptor [19]. This hypothesis is corroborated strongly by the different trends of the positive and negative branches of the $V-I$ curve, confirming the asymmetry of the electron transfer chain inside the protein [9]. Thus it is possible to induce the electron transfer even without light irradiation by applying the proper voltage between the tip and the substrate. A simple estimation of the energy associated with a photon of 800 nm (maximum of BChl absorption [10]) gives a value which is about half the value we obtained for the resonance. Therefore this result agrees with our hypothesis, as energy is required not only to induce the electron separation between the groups constituting the electron transfer chain, but also to overcome the sample–tip tunnelling barrier.

In the light, the process of charge separation inside the protein is caused directly by the incoming photons, and therefore $V-I$ measurements cannot elicit any detectable resonance. Under these conditions RC can be considered as a permanent dipole. Surface potential measurements were applied to quantify the effect of this dipole-like behaviour, namely the potential generated by the monolayer when irradiated by light (Table 1). Thus, to explain the loss of resolution in the light we settled the hypothesis that the tip could rearrange the molecules under itself due to their dipole-like behaviour induced by light.

Therefore we studied a RC monolayer fixed with gluteraldehyde (GA). First, the deposited sample was

TABLE 1. Surface potential measurements of an RC monolayer in the dark and in the light. In the case of irradiation, the measured value of 344 mV agrees with considerations of the value of the maximum photovoltage available in such a protein, which is 495 mV [9]. The marked difference between the light and dark values indicates that the protein system is working

One RC monolayer	Surface potential (mV)
Dark	25 ± 1
Light	344 ± 1

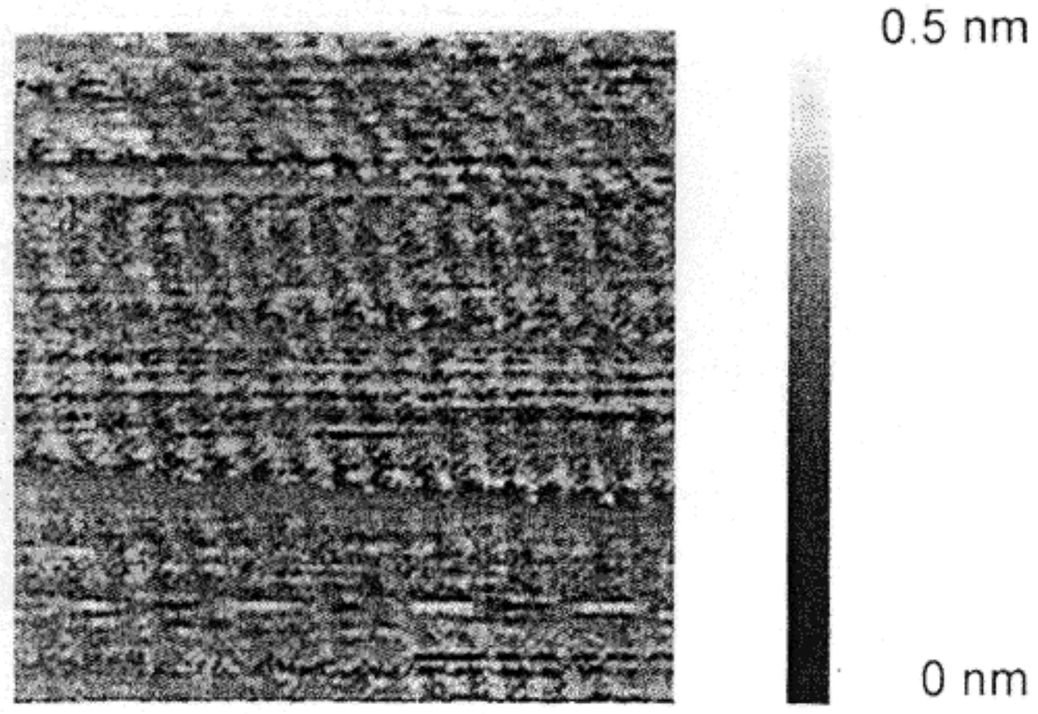
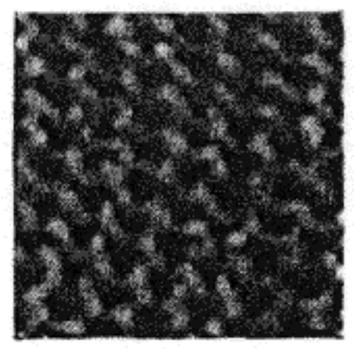


Fig. 3. STM image of an RC monolayer treated with GA (1%, 2 min) after deposition; the image (57.6 × 57.6 nm²) was acquired in the light. Scanning parameters are the same as in Fig. 1. Images, obtained in the dark and in the light, displayed the same features. Steps in the image are probably due to graphite. The lattice shows better regularity with respect to the untreated film.

immersed into 1% GA solution for 2 min, then washed and dried with nitrogen [14]. This action was expected to "freeze" the film into a rather rigid network, avoiding any rearrangement of the molecules under the tip action. Figure 3 shows an STM image obtained on the fixed sample during irradiation. The same features were imaged in the dark. The periodicity measured in this case is again 30 ± 1 Å, as in the just deposited film. However, probably due to the fixative effect, the 64 Å modulation disappears. The similarity of the images obtained during darkness and light confirms that the fixing of the molecules inside the LB film prevents the molecule's reorientation during the imaging process. Surface potential measurements on this cross-linked film give results similar to that in Table 1, confirming the protein activity (data not shown).

Afterwards we investigated the effect of heating upon the film structure [20], knowing that the protein secondary structure is preserved even at 200 °C for LB films [21]. Therefore, the sample was heated at 150 °C for 10 min and was then imaged (Fig. 4). The images obtained were exactly the same both in the dark and during irradiation. In these images, a highly increased order was evident and a spacing of 27 ± 1 Å appeared



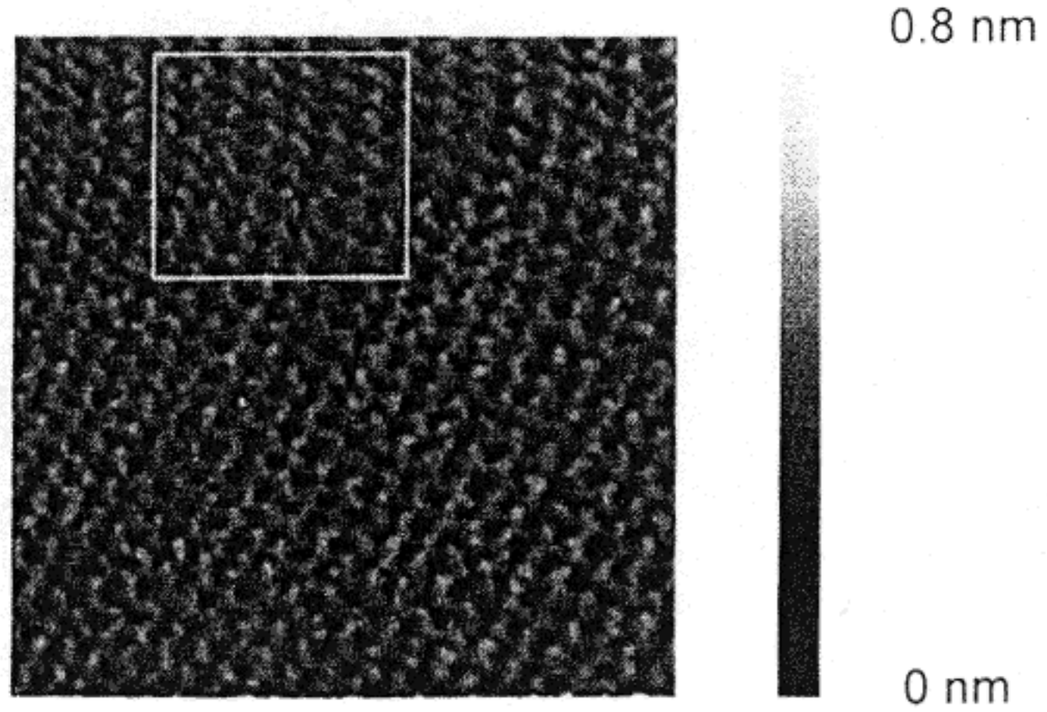


Fig. 4. STM image of an RC monolayer after heating at 150 °C for 10 min. Scanning parameters are the same as in Fig. 1. (a) Image size 57.6 × 57.6 nm²; (b) zoomed area (21.3 × 21.3 nm²) from the outlined area. Images in the light and in the dark were identical.

(see also zoomed image) together with a new hexagonal packing (according to the symmetry of the Fourier transform).

The increased order in the film structure, in comparison with that in Figs. 1 and 3, seems to be due to the closer packing of the previously identified sub-units, which have undergone a recrystallization process induced by temperature. Moreover, both in the dark and in the light, the V–I characteristics were perfectly straight lines (data not shown), indicating that the functional activity of the proteins was lost.

Surface potential measurements on the film in the dark and in the light (Table 2) show clearly that heating induces the loss of protein activity. Further, the measured values of the surface potential, being almost constant in both cases and nevertheless intermediate between the values obtained on the unheated film (Table 1), are in agreement with the hypothesis of recrystallization after heating.

TABLE 2. Surface potential measurement of an RC monolayer after heating at 150 °C for 10 min in the dark and in the light. The independence of the values upon irradiation suggests that the protein functionality has been destroyed

One RC monolayer	Surface potential (mV)
Dark	192 ± 1
Light	193 ± 1

4. Conclusion

To sum up, we have shown that STM images of LB RC films are affected by both protein structural features and protein function. The analysis of $V-I$ curves, both in the dark and in the light, indicates the activity of the single protein molecules in the film (in complete agreement with surface potential measurements at the macroscopic level).

In particular, it was found that molecules excited by light can move under the STM tip. Fixing the molecules in the film by GA prevents their reorientation.

Heating the monolayer, while destroying the protein function, improves drastically the order of the protein assembly, changing the original metastable state to a new packing of protein sub-units with a hexagonal lattice and higher density.

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References

1 A. Cricenti, S. Selci, A. C. Felici, R. Generosi, E. Gori, W. Djaczenko and G. Chiarotti, *Science*, *245* (1989) 1226.
2 R. J. Driscoll, M. G. Youngquist and J. D. Baldeschwieler, *Nature*, *346* (1990) 294.
3 H. Arakawa, K. Umemura and A. Ikai, *Nature*, *358* (1992) 171.
4 R. D. Edstrom, M. H. Meinke, X. Yang, R. Yang, V. Elings and D. F. Evans, *Biophys. J.*, *58* (1990) 1437.
5 H.-J. Butt, K. H. Downing and P. K. Hansma, *Biophys. J.*, *58* (1990) 1473.
6 H. G. Hansma, A. L. Weisenhorn, S. A. C. Gould, R. L. Sinsheimer, H. E. Gaub, G. D. Stucky, C. M. Zaremba and P. K. Hansma, *J. Vac. Sci. Technol.*, *B9*(2) (1991) 1282.
7 J. P. Allen and G. Feher, *Proc. Natl. Acad. Sci. USA*, *81* (1984) 4795.
8 C. C. Moser and P. L. Dutton, *Biochemistry*, *27* (1988) 2450.
9 A. F. Janzen and M. Seibert, *Nature*, *286* (1980) 584.
10 G. Alegria and P. L. Dutton, *Biochim. Biophys. Acta*, *1057* (1991) 239.
11 C. Nicolini, M. Adami, F. Antolini, F. Beltram, M. Sartore and S. Vakula, *Phys. World*, *5* (1992) 30.
12 Yu. Lvov, V. Erokhin and S. Zaitsev, *Biol. Mem.*, *4*(9) (1991) 1477.
13 M. Y. Okamura, A. Stainer and G. Feher, *Biochemistry*, *13* (1974) 1394.
14 V. Erokhin, R. Kayushina, Yu. Lvov and L. Feigin, *Stud. Biophys.*, *139* (1989) 120.
15 I. Langmuir and V. Schaefer, *J. Am. Chem. Soc.*, *60* (1938) 1351.
16 R. H. Tredgold and G. W. Smith, *Thin Solid Films*, *99* (1983) 215.
17 G. Feher, J. P. Allen, M. Y. Okamura and D. C. Rees, *Nature*, *339* (1989) 111.
18 H. Komiya *et al.*, Photosynthetic Reaction Centres, Pre-release 4RCR, Protein Data Bank, 9 September 1991.
19 W. W. Parson, in J. Amesz (ed.), *Photosynthesis*, Elsevier, New York, 1987, pp. 43–61.
20 L. Bourdieu, O. Ronsin and D. Chatenay, *Science*, *259* (1993) 798.
21 C. Nicolini, V. Erokhin, F. Antolini, P. Catasti and P. Facci, *Biochem. Biophys. Acta*, *1158* (1993) 273–278.