ISSN 1063-7850, Technical Physics Letters, 2019, Vol. 45, No. 10, pp. 981–983. © Pleiades Publishing, Ltd., 2019.
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Determining the Static Dielectric Constant of Individual Hemoglobin Molecules by Electrostatic Force Microscopy

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Received April 5, 2019; revised June 4, 2019; accepted June 20, 2019

Abstract—A technique for determination of the dielectric constant of individual hemoglobin molecules is presented. It is based on modeling the profiles of their images obtained using electrostatic force microscopy. The obtained values of the static dielectric constant are in agreement with the known literature data. The proposed method can be adapted to determine the dielectric characteristics of individual molecules of various proteins.

Keywords: hemoglobin, electrostatic force microscopy, dielectric permittivity.
DOI: 10.1134/S1063785019100067

Sensitivity of electrical and dielectric properties of proteins to changes in environmental conditions determines the prospect of their use as an active element of a biosensor for measuring physical, chemical, biological or any other parameters that affect the response of the biosensor in the form of the changes in capacitance, voltage, resistance or current [1]. To exclude the effects of collective interaction in an ensemble of molecules on the processes of polarization and charge transfer in protein molecules, it is necessary to develop experimental methods for determining their electrical parameters at the level of individual molecules. This is important for better understanding the effect of environmental conditions on aforementioned properties.

The choice of hemoglobin (Hb) as an object of study in this paper is motivated by the fact that it is well investigated and often used as a model globular protein. In addition, Hb is used in biosensors [2].

Electrostatic force microscopy (EFM) is a unique method for studying dielectric properties of individual micro- and nanoobjects. This method has a high sensitivity and lateral resolution and represents a powerful tool for studying the dielectric response and the distribution of the electric field and surface charge density of submicron size objects. The possibility to use EFM for quantitative determining the dielectric response of single bacterial cells with high accuracy and reproducibility was demonstrated in [3]. The purpose of this work is to develop an electrostatic force microscopy technique for determining the dielectric constant of individual hemoglobin molecules.

To study the dielectric response of individual Hb molecules, the sample was fabricated as follows. Individual protein molecules were deposited from an aqueous solution of Hb (25 μL) with a concentration of 70 μg/mL onto freshly cleaved surface of highly oriented pyrolytic graphite (HOPG). After 20 min, HOPG was washed in a deionized water to remove unabsorbed protein and dried in air.

EFM measurements of the sample were carried out using an MFP-3D SA atomic force microscope (Asylum Research, United States) at the Omsk Regional Center for Collective Use (Omsk Scientific Center, Siberian Branch, Russian Academy of Sciences). To eliminate the influence of the sample surface relief on the measurement results, a two-pass technique was used in each scan line on the first pass, the surface relief was determined using a tapping mode. On the second pass, the probe tip was removed from the surface at a predetermined distance and rescanning was performed along the trajectory of the first pass at applied DC voltage on probe tip.

The recorded signal is the phase shift of the cantilever oscillations on the second pass. The surface distribution of the phase shift forms the EFM image. The scan height in the second pass was chosen from the condition of the best signal-to-noise ratio on an EFM image that is achieved by varying the height of the tip above sample and the amplitude of cantilever oscillation. When varying these parameters in the experiment, an optimum lift height of 30 nm was determined on the second pass.

On the second pass, a constant bias voltage of +3, +5, +7, −3, −5, and −7 V was applied to the probe. Later, on the basis of the dependence of the EFM signal magnitude on the applied voltage, we determined

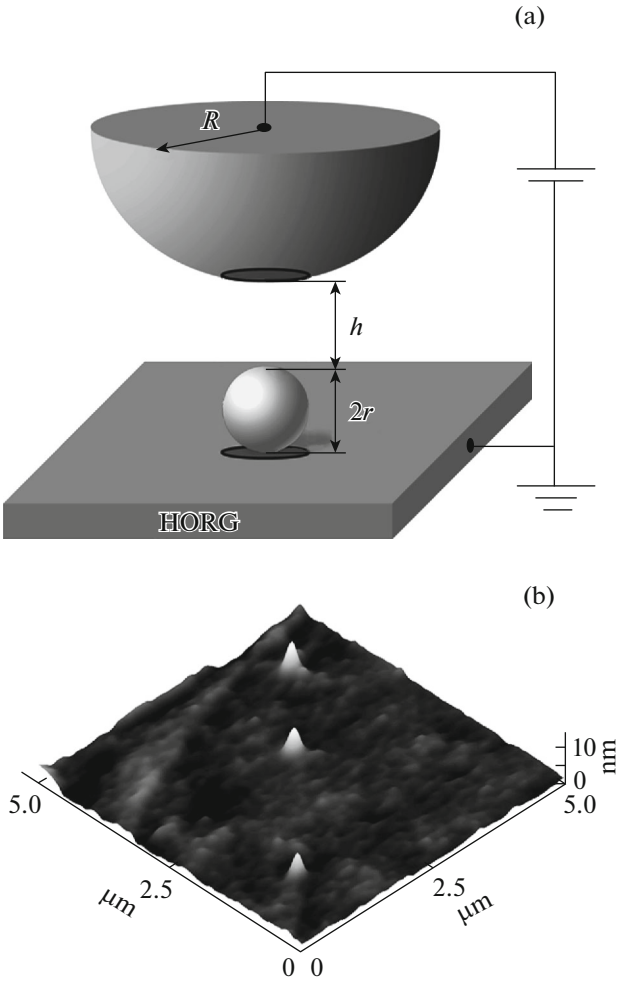

Fig. 1. (a) Schematic representation of an EFM experiment and (b) a 3D image of the HOPG surface with immobilized individual Hb molecules.

the external contact potential difference (CPD) between the probe and HOPG [4]. It is necessary to take the CPD into account to determine the actual voltage between the probe and the substrate when calculating the theoretical value of the EFM signal and accurately calculating the Hb dielectric constant.

To eliminate the effect of adsorbate on the dielectric response, all measurements were carried out in a dry nitrogen atmosphere at relative humidity RH ~ 5%. We used ETALON HA_FM conductive cantilevers (NT-MDT SI, Russia) with a Pt coating and the following parameters: resonance frequency ~100 kHz and tip curvature radius ~35 nm.

Figure 1a shows the scheme of an EFM experiment. A hemoglobin molecule having the form of a ball with radius r (within the model used) is located on the HOPG substrate. A tip with curvature radius R is located at constant height h above the sample, and constant voltage U is applied between the probe and the substrate.

According to [5, 6], the theoretical difference of the phase shifts characterizing the object under study is calculated by the formula

$$\Delta\mathbf{\Phi}_{\text{theor}} = \mathbf{\Phi} - \mathbf{\Phi}_0. \quad (1)$$

The quantities entering expression (1) are the phase shift of cantilever oscillations provided by the capacitive probe–substrate coupling,

$$\mathbf{\Phi}_0 = \arctan\left[\left(\frac{Q}{2k}\right)(U-\Delta U)^2\frac{\partial^2 C_0(z)}{\partial z^2}\right], \quad (2)$$

and the phase shift determined by the capacitive coupling between the probe and sample,

$$\mathbf{\Phi} = \arctan\left[\left(\frac{Q}{2k}\right)(U-\Delta U)^2\frac{\partial^2 C(z)}{\partial z^2}\right], \quad (3)$$

where ΔU is the CPD between the probe and sample, $Q = 234$ and $k = 3.4$ N/m are the quality factor and the cantilever spring constant, respectively, and $\frac{\partial^2 C_0(z)}{\partial z^2}$ and $\frac{\partial^2 C(z)}{\partial z^2}$ are the second-order derivatives of the correspond capacitances calculated by the formulas

$$\frac{\partial^2 C_0(z)}{\partial z^2} = 4\varepsilon_0\int_0^r\int_{y_1}^{y_2}[h+R-\sqrt{R^2-x^2-y^2}]^{-3}dxdy, \quad (4)$$

$$\frac{\partial^2 C(z)}{\partial z^2} = 4\varepsilon_0\int_0^r\int_{y_1}^{y_2}\left[h+R-\sqrt{R^2-x^2-y^2} + 2\left(1-\frac{1}{\varepsilon}\right)(r-\sqrt{r^2-x^2-y^2})+\frac{2r}{\varepsilon}\right]^{-3}dxdy. \quad (5)$$

Here, ε_0 is the electric constant, ε is the dielectric permittivity of a Hb molecule, and y_1 and y_2 are the limits of integration along the ordinate axis, namely, $-\sqrt{r^2-x^2}$ and $\sqrt{r^2-x^2}$, respectively.

The dielectric constant of Hb molecules was determined using expressions (1)–(5) and fitting the theoretical difference of the phase shifts to the experimentally measured value.

Figure 1b shows a 3D image of an HOPG surface with individual immobilized Hb molecules. The height of Hb molecules was determined from topographic images (Fig. 2a), while, to calculate the phase shift difference between the molecule and the substrate (Fig. 2c), the profile cross section on EFM images (Fig. 2b) was extracted.

To check whether the contrast of the EFM images of the Hb molecule is due to capacitive probe–sample coupling and is not connected with the presence of free charges on the protein molecule, the dependence of tanΦ on U^2 was plotted (Fig. 3). The strict linear dependence indicates that the contrast of the EFM images of Hb molecules is determined only by the

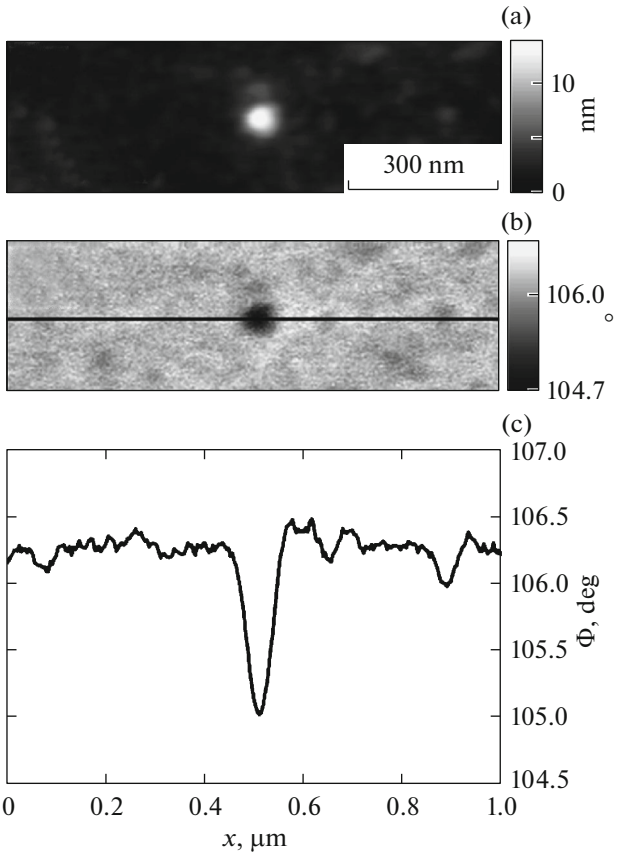

Fig. 2. (a) Topographic image of the Hb molecule; (b) the corresponding EFM image at a voltage of +7 V on the probe. (c) Cross-sectional profile of an EFM image.

probe–sample capacitive coupling, accordingly, equations (1)–(5) can be used for quantitative calculations.

Based on the profile modeling of the EFM images of individual Hb molecules, the average value of the dielectric constant of protein was determined, which, taking the spread into account, proved to be 2.9 ± 0.9. According to the literature, the dielectric constant of proteins lies in the range of 2–4 [7–9], which indicates the appropriateness of the values obtained.

Thus, this work has shown a method for determining the static dielectric constant of individual hemoglobin molecules based on a quantitative analysis of profiles of their EFM images using the model of electrostatic interaction between an atomic force microscope probe and a protein molecule. Using the technique described above, values of the dielectric constant for individual Hb molecules were obtained that are in accordance with the known literature data for proteins. The proposed technique can be used to study the dielectric characteristics of other protein molecules.

FUNDING

The work was performed within a government assignment to the Omsk Scientific Center, Siberian Branch, Russian Academy of Sciences, within the Basic Scientific Research Program for State Academies for 2013–2020 (registration number in EGISU NIOKTR system, AAAAA17-117041210227-8).

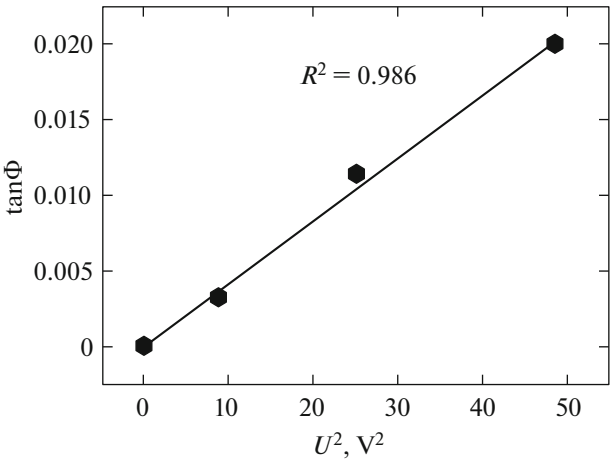

Fig. 3. Dependence tanΦ vs. U^2 for an individual Hb molecule with a diameter of 8 nm.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. F. Bibi, M. Villain, C. Guillaume, B. Sorli, and N. Gontard, Sensors **16**, 1232 (2016). https://doi.org/10.3390/s16081232
2. Yu. D. Zhao, Y. H. Bi, W. D. Zhang, and Q. M. Luo, Talanta **65**, 489 (2005). https://doi.org/10.1016/j.talanta.2004.06.028
3. D. Esteban-Ferrer, M. A. Edwards, L. Fumagalli, A. Jurez, and G. Gomila, ACS Nano **8**, 9843 (2014). https://doi.org/10.1021/nn5041476
4. N. A. Davletkildeev, D. V. Stetsko, V. V. Bolotov, Y. A. Stenkin, P. M. Korusenko, and S. N. Nesov, Mater. Lett. **161**, 534 (2015). https://doi.org/10.1016/j.matlet.2015.09.045
5. C. Staii and A. T. Johnson, Nano Lett. **4**, 859 (2004). https://doi.org/10.1021/nl049748w
6. N. A. Davletkildeev, D. V. Sokolov, I. A. Lobov, and V. V. Bolotov, Tech. Phys. Lett. **43**, 205 (2017). https://doi.org/10.21883/PJTF.2017.04.44297.16489
7. L. Fumagalli, G. Gramse, D. Esteban-Ferrer, M. A. Edwards, and G. Gomila, Appl. Phys. Lett. **96**, 183107 (2010). https://doi.org/10.1063/1.3427362
8. R. Pethig and D. B. Kell, Phys. Med. Biol. **32**, 933 (1987).
9. T. Simonson, Rep. Prog. Phys. **66**, 737 (2003). https://doi.org/10.1088/0034-4885/66/5/202

Translated by G. Dedkov