Charge transport in bacteriorhodopsin monolayers: The contribution of conformational change to current-voltage characteristics

E. Alfinito1,2(a) and L. Reggiani1,2

1 Dipartimento di Ingegneria dell’Innovazione, Università del Salento - via Arnesano, I-73100 Lecce, Italy, EU
2 CNISM, Consorzio Nazionale Interuniversitario per le Scienze Fisiche della Materia via della Vasca Navale 84, I-00146 Roma, Italy, EU

received 13 November 2008; accepted in final form 23 February 2009
published online 1 April 2009

PACS 87.85.jc – Electrical, thermal, and mechanical properties of biological matter
PACS 87.15.hp – Conformational changes
PACS 87.10.Rt – Monte Carlo simulations

Abstract – When moving from native to light-activated bacteriorhodospin, modification of charge transport consisting of an increase of conductance is correlated to the protein conformational change. A theoretical model based on a map of the protein tertiary structure into a resistor network is implemented to account for a sequential tunneling mechanism of charge transfer through neighbouring amino acids. The model is validated by comparison with current-voltage experiments. The predictability of the model is further tested on bovine rhodopsin, a G-protein coupled receptor (GPCR) also sensitive to light. In this case, results show an opposite behaviour with a decrease of conductance in the presence of light.

Copyright © EPLA, 2009

Introduction. – In recent years, several papers reported on the electrical properties of single proteins inserted into hybrid systems [1–5]. The principal aim of these researches is to explore the possibility to design nanodevices, mainly nanobiosensors, with extreme sensitivity and specificity. Most work was devoted to metalloproteins, which exhibit semiconductor-like conductivities, but increasing attention has been also addressed to bacteriorhodopsin (bR), the light-sensitive protein present in Archaea. Bacteriorhodopsin exhibits a conductivity close to that of an insulator, and is robust against thermal, chemical and photochemical degradation [3,4]. It is a protein (opsin)-chromophore complex that underlies photon-activated modifications in which the chromophore changes its structure from the all-trans to the 13-cis. The chromophore evolution induces multiple protein transformations, which fastly drive the protein from the K590 to the L550 state. In the slow transition from this state to the M410, a proton is released. Junctions, prepared with bR monolayers and contacted with Au electrodes, show non-linear I-V characteristics both in the dark (or blue light) and in the presence of green light [3–5]. It is generally assumed, that green light produces a more intense current as a consequence of the modification of the chromophore-opsin complex. Although the experimental results are not completely assessed from the quantitative side [4], the main results of available experiments can be summarized as follows. i) A conductivity, of about four orders of magnitude higher than that of a homogeneous layer of similar dielectric material, was observed. ii) The current voltage (I-V) characteristics exhibit a super-ohmic behaviour pointing to a tunnelling charge-transfer mechanism. iii) The presence of green light significantly increases the electrical conductivity up to about a factor of three with respect to the dark value. iv) The I-V sensitivity to green light is washed out by substituting the original chromophore with one that is not sensible to light. v) When the chromophore of the protein is completely removed, the conductivity is suppressed by three orders of magnitude, taking the value of a standard dielectric. Despite the presence of these experiments, the existing theoretical approaches only provide a phenomenological interpretation of some I-V characteristics in terms of metal-insulator-metal tunneling theories [5,6]. The aim of this work is to develop a theoretical model able to capture the main features of the above experiments and sufficiently general to constitute a new framework for
describing electron transport properties through proteins. To this purpose, the protein conductivity is modelled by a sequential tunneling mechanism through the protein amino acids, whose positions pertain to a specific conformation. Thus, the conductivity is directly connected to a protein tertiary structure and a light-induced structural change will result in a conductivity change which can be quantitatively determined.

The network tunneling model. – As general features, the protein electrical conductivity is close to that of an insulator, but it can increase significantly in particular environmental conditions, like in organic semiconductors [7]. Furthermore, the microscopic mechanism of charge transport in proteins is very articulated and includes electron, ion, and proton transport [8] in a yet not completely understood sequence. The difficulty to discriminate among these kinds of transport is further enhanced by the lack of experiments, mainly related to the difficulty of working with biological materials at the nanoscale. The most qualified mechanisms to describe electron transport in proteins are the hopping/tunneling processes [9,10]. In this framework, the transport model which assumes the existence of privileged pathways of sequential tunneling has gained a wide consensus [11]. By embracing this picture, we describe the protein as a network and the electron transport as due to a sequential tunneling by a thermionic-emission mechanism, in case further evidence.

At this stage, we neglect thermal fluctuations because the relevant signals of experiments do not suggest their evidence.

To the purpose of investigating the conduction properties of the protein, the graph is transformed into a resistor network: Each link is replaced by an elemental resistance, which mimics the different charge transfer property between neighbouring amino acids associated with their mutual distance. To detect the differences in conduction properties between two protein states, as those produced by the conformational change, we adopt the simplest parameter model by choosing the same resistivity for each amino acid. In this way, the conformational change and the interaction radius are the only relevant input parameters of the theory.

As elemental resistance of the network we take

\[ R_{i,j} = \frac{l_{i,j}}{A_{i,j}} \rho, \]

(1)

where \( A_{i,j} = \pi (R_c^2 - l_{i,j}^2 / 4) \) is the cross-sectional area between the spheres of radius \( R_c \) centered on the \( i \)-th and \( j \)-the node, respectively, \( l_{i,j} \) is the distance between these centers, \( \rho \) is the resistivity.

By construction, each elemental resistance depends upon the distance between nodes. Therefore, following up a conformational change, the variation of this distance implies a variation of each elemental resistance, which eventually leads to a variation of the network resistance (and thus of the protein resistance). As a consequence, a topological transformation can be monitored by means of resistance measurements.

By taking the first and last amino acid of the protein primary structure as ideal electrical contacts, the protein resistance for a given \( R_c \) is calculated by solving the corresponding linear resistance network within a standard Kirchhoff framework [14]. Being interested to a change of the resistance, rather than to its absolute value, this choice for the contacts does not modify the results of the model. Numerical calculations show that for \( R_c \) below a threshold value (typically around 4 Å) the network is disconnected and thus not conducting at all. By contrast, for \( R_c \) well above the average distance between amino acids (typically above 15 Å) [14] each amino acid is connected with most of the others, thus the conduction becomes insensitive to modification of the structure. Between these critical values of \( R_c \), the change of resistance exhibits a smooth behaviour. Accordingly, we select the value of \( R_c \) which maximizes the change of resistance due to the conformational change, and which is found to be around 6 Å, a well-accepted value for the interacting radius between amino acids [13–15].

To account for the strong superlinear \( I-V \) characteristic, the model implements a barrier-limited current mechanism as follows. At increasing voltage, each elemental resistance is allowed to take a second value of resistivity which, playing the role of a small series resistance of the network, is several orders of magnitude lower than the first one. The probability of this choice mimics a barrier-limited mechanism in analogy with the case of an organic molecular layer [6]. In this way, the initial linear increase of current with applied voltage turns into a superlinear increase, with the value of the barrier energy to be fitted by comparison with experiments. Accordingly, the stochastic selection is taken to be ruled by the direct tunneling probability\(^1\) [6]:

\[ P_{i,j} = \exp \left( -\frac{2l_{i,j}}{\hbar} \sqrt{2m(\Phi - eV_{i,j})} \right), \]

(2)

where \( V_{i,j} \) is the potential drop between the \( i \)-th and \( j \)-th node, \( m \) is an effective electron mass, here taken as that of the free electron, and \( \Phi \) is the barrier height.

We notice that the model does not take into account the protein environment mainly for two reasons. The former accounts for the fact that here we are interested only in the variation of protein response, and this should be independent of the environment, since it remains the same in the conformational change. The latter reason is

\(^1\)We remark that the modelling leaves the possibility to replace tunneling by a thermionic-emission mechanism, in case further experiments would evidence a significant temperature dependence of transport characteristics.
The network resistance at the given voltage is then calculated using the value of the network resistance at the corresponding voltage. The resistance of the corresponding network is calculated as \( R = \frac{I}{\langle V \rangle} \), converges within an uncertainty less than 1%. In this case, the initial iterations (100–2000 depending on the value of the applied voltage) are not sufficient to achieve the desired accuracy, so the second step is repeated until the resistance converges. The network is then electrically solved by using the following iterative procedure:

1. The network is electrically solved by using the average value (\( \langle V \rangle \)) of the voltage range considered (0–1 V) to obtain the resistance of the single protein at low and high voltages, which is then compared with the experimental value under green light. The value of \( \rho_{\text{MAX}} \) for all the elemental resistances is stochastically replaced by \( \rho_{\text{MIN}} \) using the probability in eq. (2) according to the local potential drops calculated in the first step; the network is then electrically updated with the new distributed values of \( \rho_{\text{MAX,MIN}} \).

2. Third, the electrical update is iterated (typically \( 10^4 \)–\( 10^5 \) iterations depending on the value of the applied voltage) by repeating the second step until the resistance of the network, taken as the average value over the iteration steps \( R \), converges within an uncertainty less than 1%. In this case, the initial iterations (100–2000 depending on voltage) contain a significant numerical noise and as such they are disregarded to avoid an unwanted drift of the average value (see fig. 1). Finally, the current at the given voltage is calculated as \( I = V/R \).

The comparison between theory and experiments proceeds as follows. On the one hand, the theoretical model describes the \( I-V \) modifications of a single protein when it undergoes a conformational change. On the other hand, the experimental results are carried out on a macroscopic sample of 5 nm width and \( 2 \times 10^{-3} \) cm² cross-sectional area. Therefore, to compare the results, we normalize the current value of the single protein in its native state at 1 V to that of the experiment in the dark. The normalization corresponds to multiplying the current of the single protein by a factor of \( 10^8 \)–\( 10^9 \). The same normalization factor is used for the case of the single protein in its activated state, which is then compared with the experimental value under green light.

Results. – Figure 1 shows a typical evolution of the average resistance of the single protein at low and high voltages. Within the required uncertainty, at both voltages the resistance is found to converge above about \( 4 \times 10^3 \)

| Entry | State | Resolution (Å) | Temperature (K) | Amino acids |
|-------|-------|----------------|----------------|-------------|
| 1FBB  | Native| 3.20           | –              | 224         |
| 1FBK  | Activated| 3.20        | –              | 225         |
| 1QM8  | Native| 2.50           | 100            | 229         |
| 1DZE  | M State| 2.50          | 100            | 224         |
| 2NTU  | Native| 1.53           | 100            | 222         |
| 2NTW  | L State| 1.53          | 100            | 222         |
| 1M0K  | K State| 1.43          | 100            | 222         |
| 1M0M  | M State| 1.43          | 100            | 224         |

Table 1: Bacteriorhodopsin PDB entries.
Fig. 1: Running average of the network resistance for $\Phi = 59 \text{ meV}$. Data are obtained with an applied voltage of 0.1 and 1 V, respectively.

Fig. 2: Network resistance as function of the applied voltage for the native (full squares) and activated (open circles) state for the case of: (a) $\Phi = 53 \text{ meV}$ and (b) $\Phi = 59 \text{ meV}$. Lines are guides to the eyes.

The comparison between theory and experiments is mostly qualitative. Overall, we are satisfied that the global behaviour, like the increase of current with illumination, and the shape of the experimental curves, is well reproduced. In details, figs. 3(a), (b) and (c) report the comparison of the $I-V$ characteristics of bR/PC junctions [3], obtained by a fine tuning of the barrier height with, respectively, $\Phi = 53, 59, 69 \text{ meV}$. For $\Phi = 59 \text{ meV}$ (fig. 3(b)) calculations well reproduce the $I-V$ characteristic of the native state, but underestimate that of the activated state. On the other hand, a further increase to $\Phi = 69 \text{ meV}$ does not fully account for the superlinear behaviour (see fig. 3(c)). Therefore, in analogy with the case of disordered organic materials [7,16], fig. 3(d) reports the results obtained by taking a Gaussian distribution of $\Phi$ within an average value of 69 meV and a dispersion $\sigma = 44 \text{ meV}$. We conclude that the results reported in fig. 3 (in particular fig. 3(d)) capture the essence of the experiments and validate the conjecture that the conformational change of bacteriorhodopsin can be reliably detected by current transport measurements in the presence and absence of light.

Remarkably, we notice that, when scaled by a factor of 25, the values used here for the electron effective mass and barrier heights are in reasonable agreement
with the values obtained within a simple direct tunneling model of charge transport like in a metal-insulator-metal structure [5]. Indeed, the factor 25 represents the mean number of sequential steps made by a carrier when going from one contact to the opposite one. Clearly, the advantage of the present model stems from the strict correlation between the microscopic structure of the protein and its macroscopic electrical property.

By construction, the present model reproduces also the details of experiments summarized in the introduction.

Theory predicts that in the absence of a conformational change the protein resistance remains the same. This is verified experimentally in several ways: i) By substituting the original chromophore with one not sensible to the light and finding that the $I-V$ sensitivity to green light is washed out. ii) By substituting the retinal with retinaloxime [3,4]. Then, green light does not modify the conformation and thus does not produce variation in the (nonlinear) current. iii) By using an artificial pigment derived from “locked” retinal. In this case the protein reaches an M state different from the wild M state and there is only a small current increase [4].

Theory predicts the presence or absence of the protein conductance on the basis of the range of interaction between amino acids. Absence of conduction is achieved by taking a small interaction radius (typically less than 3.8 Å). This is verified by the experiments carried out on proteins deprived of the retinal, where the response to an external bias is found to be a very low and completely noisy signal [3,4]. The origin of this result is interpreted as the protein loss of connectivity, i.e. as a dramatic deterioration of its structure.

Theory predicts the symmetry of the $I-V$ characteristic in sweeping from negative-to-positive bias, which is in accordance with experimental results.

To further test the physical plausibility of the model presented here, we have considered the case of (bovine) rhodopsin, a photoreceptor pertaining to the G-protein–coupled receptor (GPCR) family. Although the mechanism of photo-activation is essentially different from that of bR, this protein shares with bR a quite similar tertiary structure, i.e. both are seven-helices transmembrane proteins. This fact induced the researchers to take the bR structure as a template for reconstructing the 3D conformation of rhodopsin (and more generally of all the GPCR proteins). Accordingly, calculations have been carried out for the native and activated state of rhodopsin, whose structures were engineered as reported in [14]. The obtained results, which parallel those of fig. 3(b), are reported in fig. 4. Remarkably, for the analogous conformational change, the model predicts that rhodopsin exhibits a behaviour opposite to that of bR, i.e., in going from the natural to the activated configuration, current at a given voltage is suppressed. Physically, we believe that this opposite behaviour follows from the opposite change the retinal undergoes when absorbing a photon. In rhodopsin the retinal shape goes from bent to straight, while the reverse occurs in bR [12]. As a consequence, we arrive at the plausible conclusion that an opposite conformational change of the entire protein should imply an opposite change of its $I-V$ characteristics.

**Conclusions.** – We have presented a resistor network model implemented for a sequential tunneling mechanism to investigate charge transport, and in particular its structure dependence, in proteins. The model proves its capability to capture the correlation between a change of the protein conformation and that of its current-voltage characteristic. The theory is validated on recent experiments performed on a nanojunction filled with bacteriorhodopsin, a light-activated protein, which evidences a substantial increase of the current, at a given voltage, in going from the dark to the presence of a green light. A good agreement with experimental data is obtained when considering an interaction radius between the amino acids of 3 Å and an average barrier energy of 69 meV with a Gaussian distribution of standard deviation $\sigma = 44$ meV. Interestingly, the model is able to explain other details of experiments, such as the role played by the presence or absence of the chromophore responsible of the sensing activity, as well as it is compatible with simple direct tunnelling models developed on the metal-insulator-metal scheme. Under analogous conditions, application of the model to the case of bovine rhodopsin predicts an opposite behaviour with a suppression of the current. Confirmation of such a prediction remains an experimental challenge.

***

The authors acknowledge Drs C. PENNETTA and G. GOMILA for valuable discussions on the subject. This research is supported by EC through the Bioelectronic Olfactory Neuron Device (BOND) project.

**REFERENCES**

[1] Maruccio G. et al., *Adv. Mater.*, 17 (2005) 816.

[2] Andolfi L., Bizzarri A. R. and Cannistraro S., *Appl. Phys. Lett.*, 89 (2006) 183125.

[3] Jin Y., Friedman N., Sheves M., He T. and Cahen D., *Proc. Natl. Acad. Sci. U.S.A.*, 103 (2006) 8601.
[4] Jin Y., Friedman N., Sheves M. and Cahen D., *Adv. Funct. Mater.*, 17 (2007) 1417.
[5] Casuso I. et al., *Phys. Rev. E*, 76 (2007) 041919.
[6] Wang W., Lee T. and Reed M. A., *Rep. Prog. Phys.*, 68 (2005) 523.
[7] Baranosvki S. D. and Rubel O., *Charge Transport in Disordered Solids*, edited by Baranovski S. D. (John Wiley & Sons Ltd., The Atrium, Southern Gate, Chichester, West Sussex, England) 2006 and references therein.
[8] Warshel A. and Parson W. W., *Q. Rev. Biophys.*, 34 (2001) 563; Kato M., Pissiakos A. V. and Warshel A., *Proteins*, 64 (2006) 829; Burykin A., Kato M. and Warshel A., *Proteins*, 52 (2003) 412; Leite V. B. P., Alonso L. C. P., Newton M. and Wang J., *Phys. Rev. Lett.*, 95 (2005) 118301.
[9] Frauenfelder H. and Wolynes P. G., *Rev. Mod. Phys.*, 71 (1999) S419.
[10] Canters G. W. and Dennison C., *Biochimie*, 77 (1995) 506.
[11] Beratan D. N., Betts J. N. and Onuchic J. N., *Science*, 252 (1991) 1285; Betts J. N., Beratan D. N. and Onuchic J. N., *J. Am. Chem. Soc.*, 114 (1992) 4043; Regan J. J., Rissler S. M., Beratan D. N. and Onuchic J. N., *J. Chem. Phys.*, 97 (1993) 13083.
[12] Berman H. M. et al., *Nucleic Acids Res.*, 28 (2000) 235.
[13] Tirion M. M., *Phys. Rev. Lett.*, 77 (1996) 1905.
[14] Alfinito E., Pennetta C. and Reggiani L., *Nanotechnology*, 19 (2008) 065202.
[15] Juanico B., Sanejouand Y. H., Piazza F. and De Los Rios P., *Phys. Rev. Lett.*, 99 (2007) 238104.
[16] Bässler H., *Phys. Status Solidi (b)*, 175 (1993) 15.