

Detection of charge rearrangements of membrane proteins in three dimensions

Ph.D. Thesis

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INTRODUCTION

The distribution of ions in the electrolyte compartments separated by biomembranes, and the transmembrane potential are controlled by ion pumps and channels found in the membranes. This regulatory mechanism is fundamental in the physical processes of biological energy conversion and signal transduction. Understanding the function of ion pumps and the dynamic behaviour of the electrolyte are thus one of the most important tasks of biophysics.

The purple membrane of *Halobacterium salinarum* contains a specific chromoprotein called bacteriorhodopsin (bR). Triggered by light, the protein translocates protons from the intracellular side to the extracellular space resulting in proton asymmetry on the two sides of the membrane. The energy represented in this asymmetry is utilized in the life functions of the organism. BR is the simplest known ion pump and energy conversion system of biological origin. Because of this simplicity, the molecule serves as a model for studying the fundamental aspects of biological energy conversion.

The pumping mechanism of bR can be viewed as a cyclic process called the photocycle, initiated by the absorption of a photon inside the molecule. During the photocycle the molecule changes its conformation in several steps undergoing thermally activated intermediates. The process results in a net proton translocation through the membrane, and at the end the protein returns to the ground state. The different conformations have partly different absorption spectra, therefore the photocycle can be followed by spectroscopic methods.

In addition to the widely used optical approach, methods have been developed to detect the electric signals accompanying the operation of bR. It was found that the absorption kinetic and photoelectric data of the photocycle show a correlation in time. Based on this fact it was concluded that the photoelectric measurements can be interpreted in terms of charge motions inside the molecule assigned to conformational changes. It is important to emphasize that photoelectric measurements provide information independent of spectroscopic data.

For the description of the function of bR on the molecular level, several, partly conflicting molecular dynamics models have been developed in the near past. Common to these models, the calculations utilize information gained primarily from spectroscopic observations. This implies that the validity of the models cannot be confirmed or a model cannot be rejected on the basis of these experiments. On the other hand, photoelectric measurements performed with the so called suspension method render possible to deduce quantitative information on the molecular dipole moments of intermediates of the photocycle. This means that the intermediate structures can be tested directly by calculating their dipole moments and hence the corresponding molecular dynamics model can be verified.

The main goals of this work are to establish a theoretical background for the suspension method (gel method) developed for studying ion pumps, to discuss the quantitative data evaluation, and to interpret the macroscopically detectable photovoltages from the molecular level of intramembrane charge rearrangements.

For an exact discussion proper knowledge is needed on the ionic relaxation triggered by intramembrane charge motions. In order to solve this problem, i.e. to model the dynamic

behaviour of the electrolyte, a modified Brownian dynamics model and its implementation has been developed. The simulation system has been tested by comparison with the Debye-Hückel theory based on modeling the shielding effect of the ion atmosphere around an ion. After establishing a quantitative agreement between the theory and the model, the simulation system has been applied to study ionic relaxation processes. I derived the speed of ionic relaxation following an abrupt intramembrane dipole change (charge jump) in a membrane disk, both in the case of a dipole change parallel to the membrane normal, and in the membrane plane. Based on the simulations, I concluded that the ionic relaxation is kinetically anisotropic (the relaxation triggered by a dipole change in the membrane plane is faster). I showed that the anisotropy also exists in the absence of the diffuse double layer around the membrane disk, but the presence of the diffuse double layer significantly increases the effect. From additional simulations further properties of the ionic relaxation could be derived, such as the linearity of the response of the electrolyte to the dipole change, and the insensitivity of the relaxation kinetics to the geometric realization of the intramembrane dipole. The above properties of the ionic relaxation (speed, anisotropy, linearity and insensitivity to geometric details) made it possible to establish a quantitative theory for the suspension method. The evaluation of experimental data utilizing the theory of the suspension method renders possible to verify molecular dynamics models.

This work also includes a direct application of the suspension method: the investigation of two molecular dynamics models of bR, based on the photoelectric measurements. Dipole moments corresponding to the intermediates of the photocycle have been calculated both from the molecular structures given by the two models, and based on the photoelectric measurements. A noteworthy correlation existed between the dipole

moments of one of the models and the experimental values. Knowing that several factors can contribute to the uncertainty of both the theoretical and experimental values, I interpret the results very positively. It is assumed, that by further improvement of both the molecular modeling and the measuring techniques, this method will provide a decisive test for choosing the correct molecular dynamics model of bR. Such a model would be of elementary scientific importance, since it would represent the description of an ion pump on the submolecular level.

The main purpose of this work is to establish the theory of the suspension method based on dynamic modeling of ionic relaxation processes. At the same time, I consider the realization of the model and its implementation as an important result in itself, since its applicability goes beyond the problem of ionic relaxation. Due to its flexibility, the simulation system represents a general tool to investigate further phenomena of electrolyte dynamics on the mesoscopic scale.

RESULTS

1. Theoretical establishment of the suspension method.

- a. I gave a quantitative theoretical description of the physical connection between the macroscopically detectable (photo)voltages and the molecular charge motions generating them in the abstract case of the “sample of perfect direction” (II).
- b. Based on point a, I discussed the different cases of sample anisotropy corresponding to the different orienting/aligning techniques and the possibilities of using photoselection (II).

2. Photoelectric measurements on bacteriorhodopsin in three dimensions.

- a. I created a special software to support the design and interpretation of measurements with the three-dimensional suspension method (II).
- b. Based on point a, I defined an experimental technique for the exact rotational positioning of the sample (i.e. the orientation/alignment axis in the sample) relative to the measuring electrodes utilizing the photoelectric voltages directly (II).
- c. Based on point a, I described a measuring technique for the detection of the $\mu^y(t)$ component in the case of samples not perfectly oriented (II).
- d. I participated in the design and realization of the photoelectric measurements performed on bacteriorhodopsin, the evaluation of experimental data and its application to test molecular dynamic models (I).

3. Ionic relaxation – modell and implementation.

- a. I defined a modified Brownian dynamics model to model the dynamics of the electrolyte on the mesoscopic scale, and developed the corresponding software implementation. The model system contains a membrane disk (configurable with surface charges and intramembrane dipoles) and the electrolyte surrounding it in a simulation volume having diffusional walls. All physical parameters of the model system can be modified in the implementation. I created “virtual measuring devices”, which I used to perform measurements on the simulated electrolyte. The simulation tool provides a three-dimensional view to inspect the components and changes (membrane, surface charges, intramembrane dipoles, ions, virtual measuring devices) of the model system (III).
- b. I verified the solidity of the model (and implementation) by a comparison with the Debye-Hückel theory of the electrolytes at the same parameter set (electrolyte composition, temperature, simulation parameters) as used later for the dynamic investigations of ionic relaxation processes. I found a quantitative agreement of the Debye-lengths given by the theory and calculated from the simulations. (III).

4. Ionic relaxation - simulations.

I simulated ionic relaxation processes following abrupt (on the time scale of the relaxation) intramembrane charge rearrangements. By modeling the response of the electrolyte to an intramembrane dipole change created in a membrane disk, I derived the following:

- a. I determined the half time of the ionic relaxation in case of an intramembrane dipole change parallel with the membrane normal and in case of a dipole change perpendicular to it (i.e. in the membrane plane) at a given biomembrane-electrolyte configuration (geometry, surface charges, electrolyte composition, temperature). (III).
- b. Based on point a., I found that the ionic relaxation is kinetically anisotropic: the relaxation generated by the intramembrane dipole change in the membrane plane is significantly faster than in case of a dipole change parallel with the membrane normal (III).
- c. I showed that although the kinetic anisotropy of the ionic relaxation can be observed even if there are no surface charges on the membrane disk, i.e. in the absence of the diffuse double layer, the presence of the double layer around the charged membrane significantly raises the extent of anisotropy (III).
- d. I demonstrated that the kinetics of the ionic relaxation is linear with regard to the magnitude of the intramembrane dipole change in a range that contains the measuring conditions of the suspension method (III).
- e. I showed that the relaxation kinetics is insensitive to shifting the intramembrane dipole grid on a molecular distance scale. As an important implication, the concrete molecular realization of the dipole change does not affect the speed of the relaxation process, and hence, according to the theory of the suspension method, the shape of the resulting macroscopic voltage is also unaffected, which allows to establish a quantitative connection between the measured data and the molecular dipole moments (III).

PUBLICATIONS

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