Application of the maximum entropy method to analyse absorption kinetic processes

Abstract of the PhD thesis

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1 Introduction

One of the main themes of the photosynthesis research over the past 40 years was the light driven proton pump built in the *Halobacterium salinarum*. In this bacteria the light utilizing system can be found at a well-defined place at the so-called purple membrane which is built up only from some lipid molecule and from only one kind of trans-membrane protein, from the bacteriorhodopsin (BR). This protein exploits the energy of light and works as a „simple” proton pump, which transports $H^+$ ions from the cell outside the extracellular field. The proton gradient generated this way is used later for movement (phototaxis) or for ATP synthesis, while in plants this process is more complex.

After absorption of the photon the BR starts a reaction cycle, which finally returns to normal state. Such cycles are called the photocycle of BR. The different intermediates, which are formed during photocycle, have different absorption properties and this makes it possible to track the process of photocycle. Out of the normal state has been identified 5 spectrally different intermediates: K, L, M, N, O. The first model of photocycle has been reported as one-way reaction series of these intermediates. Subsequent studies have shown that although this model contains all spectrally distinguishable intermediates, the processes are much more complicated. For example one of the most important point is the M state(s) rise and decay, when the $H^+$ ion submission happens. This is a "switch" step, which allows the Schiff-base reprotonation from the cytoplasm via the Asp-96 amino acid. From detailed analysis of this intermediate soon turned out that there are several forms of M state, which are spectrally identical (at least 2: $M_1$, $M_2$, but already about the 3rd M status were also found some evidence: $M_2'$).

This resulted that the serial reversible photocycle models with more than 5 intermediate states have become more popular, which contains already more M, N or O intermediate states and explained properly the experimental data. Apart from this, other models were also appeared to explain the several M states, for example some suspected heterogeneous bacteriorhodopsin normal state, or parallel photocycle. Although during the time, when I was working on these thesis there was not available any photocycle model, which could have been acceptable from every aspect.
The properties of bacteriorhodopsin are tried to be exploited nowadays to create optical chips. In this applications of the protein plays an important role the refractive index of it, and also the change of it due to the light. These effects have already been studied in many measurement method: based on the definition of critical angle method, based on space and spectrally resolved interferometry (SSRI) method. It was made also some experiment with optical waveguide light-mode spectroscopy (OWLS), but only with thick samples.

2 Objectives

Since for the kinetics of bacteriorhodopsin photocycle exists quite a lot of models, so one of my specific goal was to introduce a new method that could help to get closer to the reality on a more independent way.

First I presented the optical spectrometer, which was used to record the absorption kinetic data during photocycle and the preprocessing method of raw measurement data. Additionally I report about the results and disadvantages of the earlier applied methods to find a kinetic model based on absorption signal measurement. Then I introduce the Maximum Entropy Method (MEM) as an alternative method for setting up the kinetic models: during it I show a detailed descriptions of the method, the earlier usage of it. I present the generalization of it, which makes it suitable for analysis of absorption kinetic measurements, as well as the testing of the method. Finally, I show, which specific photocycle model is supported by the MEM.

Another part of my work was to examine light recovery proteins (beside the BR I used light harvesting protein complex from plants: LHCII). Here, the main goal was to determine the optical parameters (refractive index, thickness) of these proteins during their light recovery.

In the other part of my dissertation I briefly present the OWLS device and traditional measurement methods and also a novel method to process the measurement data to get the BR refractive index and thickness.
3 Applied methods

The bacteriorhodopsin photocycle examination was carried out using an optical spectrometer. The sample was light through on different wavelengths and also excited by Nd-YAG laser pulses. This way was recorded the absorbency change signals from few tenths of a µs by s range with help of two DSA (Digital Storage Adaptor). The two DSAs were set with different time resolution and from those were compiled 200 points, which contained the absorption data evenly on a logarithmic scale.

Novel analysis of the absorption measurements were performed using the Maximum Entropy Method. Although MEM was originally developed for reconstruction of astronomical images, it has already been successfully used in several other disciplines, such as: high time resolution fluorescence spectroscopy, determining the speed of ligand binding. The method maximizes the entropy can be calculated from experimental data, while minimizing the deviation of the fit to experimental data. In other words it selects only one specific fit out of all the good fitting solutions. The MEM gives an opportunity to determine from experimental data the apparent rate constant behind the process, without presupposing any kinetic model. Since the method had been used in the past only for processes containing just rising or just falling rate distributions, we have to make it capable to process experimental data, which contains also rising and falling rate constants at the same time, as in case of the absorption kinetic signals of BR.

For the thickness and refractive index measurements OWLS was used. The two essential elements of the OWLS sensor is typically a few hundred nanometers thick substrate from glass and a waveguide layer (dielectric films) with high refractive index, which contains an optical grid on its surface. The light can penetrate and spread in the dielectric film only at well-defined angle when illuminating it. Since this wave is evanescent, therefore, the wave propagation is sensitive to the film boundary. This allows to use the waveguide sensor as surface detector and makes it possible to determine the optical parameters of material added to it. At small thickness of BR adlayer the calculation method used in the past didn’t give feasible result for refractive index of BR, therefore, an inverse method was developed. Supposing the optical waveguide structure is known (each
layer refractive index and thickness are known), based on the continuity criteria of electric and magnetic field strengths coming from the Maxwell’s equations the effective refractive index can be calculated with high precision with help of numerical methods. Then, change the sample thickness and refractive index value during the numerical calculation, whereas the measured effective refractive index values won’t be returned. This way can be measured and calculated the refractive index and thickness of thin BR and LHCII adlayers as well.

4 Abstract

(1) Our work began with the analysis of BR photocycle. First we have shown that the maximum entropy method which was applied earlier only for lifetime distributions containing only positive amplitudes, can be successfully adapted to absorption kinetic measurements, which contains also positive and negative amplitudes. We have proved on generated data, that intrinsic and noise contribution to the entropy can be separated, noise contributes essentially to the entropy only in that cases when the rate distribution is narrow [1]. With help of generated absorption data on several different wavelengths we proved that the MEM can determine consistently the main characteristics of a kinetic model as well [2].

(2) We analyzed real photocycle measurements by the MEM and it was found that the kinetics of BR has broad lifetime distributions, which is a sign of big „intrinsic” entropy. The inherent entropy may be connected with the protein dynamics and heterogeneity. This is an important evidence to conclude that the rate constants can be described by discrete models only as a fair approximation of the photocycle [1, 2].

(3) Despite the foregoing conclusion, I tried to find a discrete photocycle model, which characteristic rate constants fit the best the MEM results done on different wavelengths measured absorption signal. First of all, we have shown that at least 8 intermediate states exist on wide pH range in BR photocycle, which supported that time by other published models with eight intermediate states. After trying a lot of model, a branched kinetic model containing 4M state was fo-
and as best fit the results of the MEM. The legitimacy of this results of the MEM is supported also by the goodness of obtained rate constants Arrhenius representation and by direct calculation of the concentration of the intermediate states [2].

(4) During the rest of my work the optical parameters of purple membrane and LHCII adlayers over wide range of thickness (20 nm to several hundred nm) was investigated using OWLS technique. We have shown that the approximate method used previously, the 4-layer modes equations give a high error when calculating the optical parameters of purple membrane adlayers. An inverse method was developed to resolve this problem and further measurements supported its applicability to samples with smaller thicknesses. [3].

(5) With help of this method I measured the purple membrane \( (n_A = 1 : 53 \pm 0.01) \) and LHCII \( (n_A = 1 : 54 \pm 0.01) \), refractive index, which matches well with the existing literature [3].

(6) I studied the reversible and irreversible effects by illuminating purple membrane and LHCII adlayers with two laser lights. The parameters (refractive index, thickness and absorption) of purple membrane adlayers have changed only in small amount, but with the help of lock-in technique it was possible to measure \( \Delta n \approx 0.002 \) refractive index increase and \( \sim 34 \) nm thickness decrease [3]. I measured also the irreversible refractive index changes after longer illumination of LHCII adlayer, which origin can be thermo-optical.

5 Conclusions

The photocycle can not be described purely by reversible kinetic model: reality is more complicated and the refractive index of the purple membrane is clearly changes in the M state.
References

