THz differential spectroscopy of Rhodopsin

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***Abstract*— In this work we record THz spectra of Bovine Rhodopsin Rod Outer Segment (ROS) suspended in buffer. THz difference spectra (calculated using spectra collected before and after light excitation) were used to identify signals arising from the domain structural transformations which occur during the light-activation process. The Molecular Modelling Toolkit (MMTK) software package was used to calculate the theoretical normal mode density for both dark-state and light-activated rhodopsin. The simulation results were used to guide the interpretation of spectra observed.**

I. Introduction And Background

In recent years, there has been considerable interest in studying low frequency collective vibrational modes of proteins[1]. The low frequency vibrational modes can provide detailed information about a bio-molecule's conformational change via ligend binding, especially for large size molecules like proteins. Theoretical calculations predict that proteins have a multitude of low vibration modes in the far infrared range and most of the low vibration modes locate in the THz range (0.1-10THz)[2]. The low vibrational characteristics of protein can be investigated by THz domain spectroscopy (0.1-10THz) which is becoming a powerful tool in biological applications.

In this work we have studied Bovine Rhodopsin which is a photoreceptor located in the discs of rod cells that mediates vision under scotopic conditions. Bovine rhodopsin is the best characterized member of the large family of cell surface receptors called G-protein Coupled Receptors (GPCRs)[3]. The photoreaction is initiated when rhodopsin is illuminated by light (wavelength >495 nm). In our TDS THz experiments we measure the THz spectrum before illumination, corresponding to the "dark-state" of Bovine Rhodopsin, immediately after illumination, corresponding to Metarhodopsin (I&II) and more than 30 minutes after illumination corresponding to the "final state" which is predominantly a mixture of all-*trans* retinal and opsin with a marginal amount of Metarhodopsin remaining (~6%). We also present the theoretical calculation of normal modes density on active and inactive rhodopsin (dark-state), using the Molecular Modelling Toolkit (MMTK) software package[4]

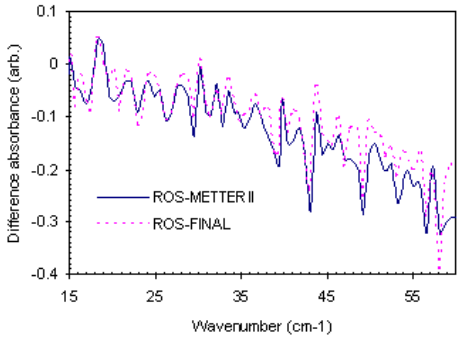
II. EXPERIMENT PROCEDURE

Bovine rhodopsin sample was purified from rod outer segments (ROS) which were prepared from bovine retinae using sucrose density gradients. The time domain THz pump-probe system set-up used in this experiment is a standard design[5]. The buffered ROS suspension (~10mg/ml) was loaded in the liquid cells with TPX windows, which allow 95% of the THz radiation to be transmitted through the cell. The path length is 15 μm in order to reduce the THz absorption by the water. The window aperture is 25 mm. Five spectra were recorded for each sample cell before illumination, shortly after illumination, and more than 30 minutes after illumination. Three measurements were repeated. The fifteen scans for each state were averaged and difference spectra were obtained

III.EXPERIMENT RESULTS

Figure 2 shows the difference absorbance spectra of dark-state-to-intermediate state and dark state-to-final state. There are few differences between the two difference absorbance spectra. There was a broad change of difference absorbance toward higher frequency band.

Figure 2: Difference absorbance between dark state and Metarhodopsin II of bovine rhodopsin.



This is similar to the results on bacterhodopsin[6]. Several reproducible peaks were observed. Some of them could be assigned. It was noted that there is a negative oscillation band located abound 43cm^{-1} in the absorbance difference spectra of rhodopsin/metarhodopsin II. This feature is speculated to correspond to the vibrational band of All-trans-retinal which is observed in ref [7] since All-tran-retinal is an isomerized product of the Metarhodopsin II. There is another negative oscillation band is 57cm^{-1} in the absorbance difference spectra. Wang et al observed a coherent oscillation at 60cm^{-1} using time resolved spectroscopy in fs time scale[8]. It originates from a torsional mode of the chromophore and with a large projection along the $C_{11} = C_{12}$ torsional mode. It is speculated that the negative oscillation band around 57cm^{-1} in the absorbance difference spectra may correspond to these torsional modes. Besides these two main bands, there were several weak oscillation features which had no corresponding resolved structure. It is suggested that these oscillations may reflect the conformational change of the protein or interaction between both protein and retinal. Above 60 cm-1, other features were

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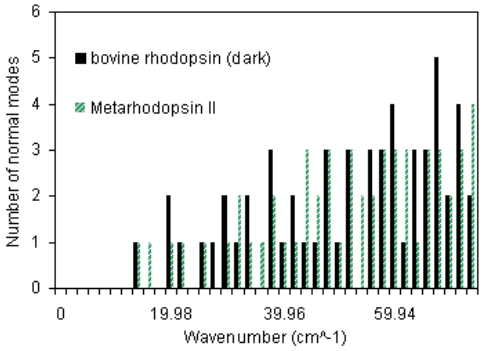
not observed because of the spectral band limit of the THz TDs used in the experiment.

Figure 3: Calculated normal modes densities of inactive and active rhodopsin based on approximation method of MMTK.



Normal mode analysis is a useful tool for studying the dynamics of biological molecules such as low frequency collective modes of proteins. Recently this method was used to predict the low frequency absorption spectrum and conformational change for both wild type and D96N mutant bactriarhodopsin[6]. The calculation showed that the absorbance increased rapidly along with the increase of frequency. This result was consistent with the related experiment work based on THz TDS. Hinsen reported a useful approximation technique applied to reduce the calculation speed for normal mode analysis based on MMTK[4]. By using this method, the calculation time for a large protein which contains more than 2000 atoms can be reduced from hundreds of hours to several tens of minutes in a personal computer. The density of normal modes of bovine rhodopsin (dark state) and Meta II, two states of bovine rhodospin during the photo bleaching process, were calculated by using approximation method and MMTK program. The atomic coordinates used in the calculations came from the Protein Data Bank. The entry code for bovine rhodopsin (dark state) was 1JFP and for Metarhodopsin was 1LN6. The calculated normal modes for both the bovine rhodopsin and Metarhodopsin are shown in *Figure 3*. As seen in *Figure 3*, the number of normal modes increases as the frequency increases. The difference between the bovine rhodopsin and Metarhodopsin II, becomes significant at high frequency band. Recently, Balu *et al* suggested that the vibrational modes at low frequency band are related to the molecular domain motion within the extra-cellular loop and the higher frequency band modes corresponds to motion in the cytoplasmic loops[9]. The negative difference of dark state-to-metar II appears at around $37cm^{-1}$ and $57cm^{-1}$ which may respond the negative peaks appeared in the experimental results.

III.CONCLUSIONS

In our experiment, we present the difference spectroscopy of purified bovine rhodopsin in water solution using time domain THz spectrometry. Three sample cells were loaded with rhodopsin in aqueous solution. We took five time domain scans for each sample cell separately in the dark state, intermediate and the final state. The difference spectra between the dark state and the intermediate state showed several oscillation features. Some of them could be assigned. Normal mode analysis was used to calculate the theoretical Normal mode density The simulation results were used to guide the interpretation of spectra observed.

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