Observation of a terahertz laser-induced Stark Effect in proteins

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Introduction

Proteins undergo a wide variety of motions, from atomic vibrations on the sub-picosecond time scale to slow folding processes in the order of seconds. Currently, biophysical methods do not permit the real-time detection of such conformational rearrangements.

The method proposed allows for the observation of side-chain fluctuations in the picosecond (ps) time scale as a function of an applied external electric field (EF). When the light hits the crystal and the EF traverses it a particular moment in time, small fluctuations in electronic configuration of the protein can be observed using spectroscopy. This technique was implemented first using green fluorescent protein (GFP) crystals, but could potentially be applied to a more generic protein such as CypA.

Why GFP?

• The large dipole of the protein chromophore should give rise to a large spectroscopic signal, and therefore easier detection;
• Literature and Stark effect studies previously performed on GFP provide a theoretical background from which to understand observed effects.

Why CypA?

• Many proteins contain one or more tryptophan residues — CypA contains one Trp.
• The location of the chromophore, Trp, can easily be modified through mutation, and the protein’s intrinsic EF can thus be assessed by looking at Stark measurements of different CypA mutants.

Keywords: electric field, GFP, CypA, electronic perturbation, Stark effect.

Methods

1. GFP Purification
   a. Large scale expression using E.coli — expression strain BL21 (DE3)
   b. Ammonium sulphate cut (45%)
   c. Anion exchange: Q-Sepharose
   d. Size exclusion: HiLoad Superdex 75;

2. GFP Crystals:
   a. Tray setting varying PEG concentration (4, 6, 8, 10 %) and GFI concentration (8, 10, 12 mg/mL)
   b. Serial dilutions of seed stocks from crystals grown at high PEG concentrations were used to obtain large, single crystals

3. CypA Double Mutagenesis

<table>
<thead>
<tr>
<th>Mutant</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>W121F</td>
<td>F8W</td>
<td>F22W</td>
<td>F36W</td>
<td>F112W</td>
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4. CypA Purification
   a. Large scale expression using E.coli — expression strain BL21 (DE3)
   b. Cation exchange: HiTrap SP XL
   c. Anion exchange: MonoQ
   d. Size exclusion: HiLoad Superdex 75 pg

Results

GFP was expressed and purified (Figure 4a). Optimal conditions for crystal growth were found to be low PEG concentration (4–6 %) and low protein concentration (R10 mg/mL), with seeding at 10,000-fold dilution.

A spectroscopic signal was detected up to 10% at ~510nm probe light (Figure 3), with protein perturbation being dependent upon the crystal’s orientation and polarization (data not shown). The Stark effect was observed as the change in absorption energy of the chromophore (ΔE) in response to the applied EF. The change in absorbance of GFP at a given wavelength is correlated to the electric field applied. This technique was found to decrease deconstruction of the crystal due to crystal heating, as the electric field was delivered in pulses each ~10 ps long.

CypA double mutants were successfully produced and the WT protein purified (Figure 4b and 5).

Discussion

The method described represents a new technique to measure the effect of perturbation of the protein by an EF through observation of the electronic Stark effect. The signal observed indicates the perturbation of GFP due to the interaction of the dipole of the protein’s chromophore with the applied electric field, leading to the modulation of charge within the protein. The effect observed can in principle be used to calculate the effective EF delivered to the protein. It will be interesting to see whether these effects can be understood in the context of changes in the protein structure.

We also began studies to test the validity of the technique on a generic protein. In the future, CypA crystals will be used to perform measurements to observe the Stark effect and to compare spectroscopic changes between mutants. Hypothetically, mutations will lead to Stark shifts of varying amplitude that can be detected and interpreted in terms of changes in the protein’s internal EF based on the location of a given Trp mutation.

Conclusion

This project has identified a new technique which utilises an electric field to induce small conformational changes in green fluorescent protein crystals. Further studies could extend the use of this setup for more generic proteins to investigate their structural changes on the picosecond time scale.

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