Green Fluorescent Protein Hydrogen Bonds Compared to Microelectronic Devices

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Abstract – **Green Fluorescent Protein hydrogen bonding network in ground state is investigated. The network consists of chromophore, water molecule, and protein residues around the chromophore that participate in the photocycle. Proton transfer characteristics for each hydrogen bond are obtained. It is found that the proton transfer parameter depends on donor and acceptor electrostatic potentials, cooperative effects, and the sum of protein electrostatic potentials. The shapes of proton transfer parameters versus donor and acceptor electrostatic potentials are similar to *I-V* characteristics of 2- or 3-terminal devices. In addition, there are characteristics that are similar to reverse diode characteristic, output characteristic of field effect transistor, current source. The chromophore and glutamine acid residuum in hydrogen bonding networks have functions similar to microelectronic multiplexer.**

Keywords – **Hydrogen bonding networks, green fluorescent protein, proton transfer characteristics.**

I. Introduction

Green fluorescent protein (GFP) is one of the most wide spread proteins. It has application to biology and medicine as a biological marker, in bioelectronics – for biosensors [1], for photodiodes [2]. Its chromophore and fluorescence properties are the basis for GFP applications. During the photocycle, in ground state A the chromophore is neutral and can be photoexcited at 397 nm to state *A**. State *A** evolves very rapidly to an intermediate state *I** with decay times on the order of a few picoseconds. State *I** can either decay to the ground state *I* (and later revert back to *A*) or it can further evolve to state *B**, eventually relaxing to *B* [3]. The chromophore is bonded to a hydrogen bonding network (HBN). The HBN plays crucial role in the overall photocycle and in chromophore stabilization.

In the present paper we will investigate the proton transfer via hydrogen bonds formed around and with the chromophore. We will also examine how the proton transfer depends on donor-acceptor potentials, and surrounding residues. We will compare the proton transfer characteristics to the characteristics of known microelectronic devices to find its potential applications in bioelectronics.

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II. Materials and Methods

We will use a wild type green fluorescent protein in A state (neutral chromophores). The high-resolution crystallographic structure of GFP (2wur) is taken from Protein Data Bank [4]. It is made by X-ray diffraction at resolution of 0.9 Å. The chromophore-atom HBN is visualized by Vega ZZ [5]. The distances between hydrogen bond donors and acceptors are measure by Vega ZZ.

The pH-dependent electrostatic potentials $\Phi_{el,i}$ and the *pKa* of ionizable groups are calculated by PHEI server [6] using the followoing equations:

$$\Phi_{el,i}(pH) = 2.3RT\sum_{j\neq i}\{Q_j(pH)W_{ij}[1-(SA_i+SA_j)/2]\} \quad (1)$$

where $Q_j(pH)$ is defined by degree of dissociation or statistical mechanical proton population of given H+-binding site; W_{ij} is pair-wise interaction; *SA* is solvent area.

The pKa is calculated by

$$pKa_i(pH) = pKa_{\text{int}i} + \\ +\frac{1}{2.3RT}\sum_{j\neq i}\{Q_j(pH)(W_{ij}-C)[1-(SA_i+SA_j)/2]\} \quad (2)$$

where R – gas constant, T – temperature in Kelvins, pKa_{int} is the *pKa* of the *i*-th site according to model compounds.

In this way we calculate the electrostatic potential of each participant in the HBN. Using the calculated *pKa*-s we can also calculate the bottom of potential wells of each donor and acceptor; these bottom potential wells are needed of the calculation of proton transfer *K*.

For the calculation of proton transfer *K*, we have developed a custom code that is based on Marcus parameterization [7]. In this parameterization, the cooperative effects and surrounding residue electrostatic effects are taken into account. The *K* parameter is calculated by:

$$K = \frac{k_B T}{2\pi} \exp(-\frac{Eb - h\omega/2}{k_B T}) \quad (1)$$

where: K – proton transfer parameter, k_B – Boltzmann constant, Eb – energy barrier, h – Planck constant, ω – frequency, T – temperature [°K].

The energy barrier is calculated by:

$$Eb = (s_A(R(DA) - t_A)^2 + v_A) + s_B E_{12} + \\ + (s_C \exp(-t_C(R(DA) - 2)) + v_C)(E_{12})^2 \quad (2)$$

where $R(DA)$ — distance between the donating and accepting atoms, E_{12} — energetic difference between bottom potential wells donating and accepting atoms; the values of other parameters are taken from [7]. The proton transfer parameter is measured in [J/mol] and the proton current is proportional to K.

It should be noted that the higher the K parameter, the easier the positive charge (proton) transfer is. Therefore, with higher K we obtain larger current.

III. Results and Discussion

Green fluorescent protein hydrogen bonding network with a chromophore is shown in Fig. 1. The distances between each donor and acceptor of hydrogen bonds are given in Table 1.

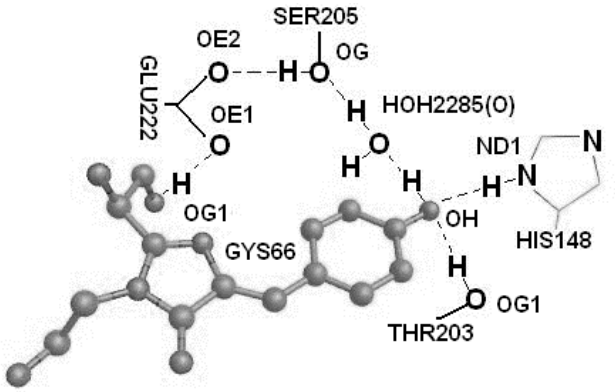

Fig. 1. Hydrogen bonding network : OH and OG1 – oxygen atoms of chromophore GYS66, ND1 – nitrogen atom of Histidine residue HIS148, OG1 – oxygen atoms of Threonine residue THR203, O – oxygen atoms of water molecule HOH2285, OG – oxygen atoms of Serine residue SER205, OE1 and OE2 – oxygen atoms of Glutamic acid residue GLU222.

Table 1. Distance between donor and acceptor of hydrogen bonds

Hydrogen Bond	Distance [Å]
HIS148(ND1)…(OH)66GYS	2,79
THR203(OG1)…(OH)66GYS	2,69
GYS66(OH)…(O)2285HOH	2,66
HOH2285(O)…(OG)205SER	2,63
SER205(OG)…(OE2)222GLU	2,59
GYS66(OG1)…(OE1)222GLU	2,78

In Fig. 1, the chromophore atom (OH)66GYS is proton donor of caged water molecule HOH2285. From the other hand, it is a proton acceptor in the hydrogen bonds with (ND1)148HIS and (OG1)203THR. The water molecule is also proton donor of SER205 which is on its turn a proton donor of the strong proton acceptor GLU222. Note that in addition GLU222 is acceptor of the chromophore atom (OG1)66GYS.

For the purposes of our paper we will investigate only this part of the HBN (close to the chromophore) although the HBNB is branched till the periphery of the protein including tens of residues (not showed in Fig. 1).

After determination of the participants of the HBN around the GFP chromophore and their donor/acceptor role, we start to investigate the characteristics of the hydrogen bonds. For this reason we vary the pH of the environment around the entire protein. The pH variation initiates polarization and ionization of the groups. Immediately, the charges of the system of protein residues, chromophore and water molecules are redistributed and the potentials of all atoms are changed (including investigated donor and acceptor atoms from target HBN). Subsequently, changing the charges result in change of the proton transfer conditions. The proton transfer parameters (K) versus electrostatic potentials (El. pot.) of hydrogen bonding donors and acceptors are shown in Fig. 2 to Fig. 4.

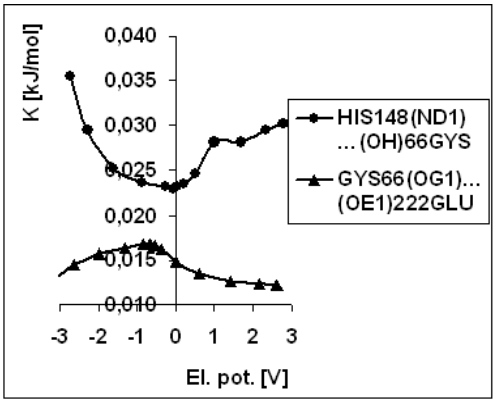

Fig. 2. Proton transfer parameter (K) vs. electrostatic potentials (El. pot.) curve of HIS148(ND1)…(OH)66GYS and GYS66(OG1)…(OE1)222GLU hydrogen bonds.

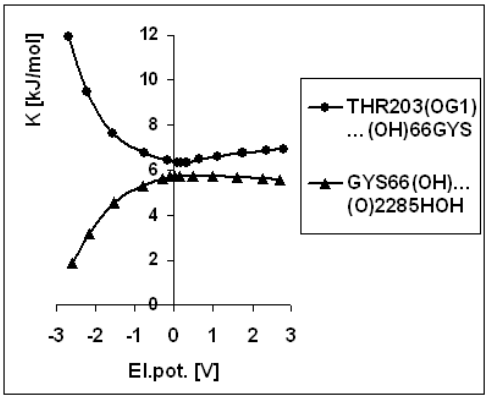

Fig. 3. Proton transfer parameter (K) vs. electrostatic potentials (El. pot.) curve of THR203(OG1)…(OH)66GYS and GYS66(OH)…(O)2285HOH hydrogen bonds.

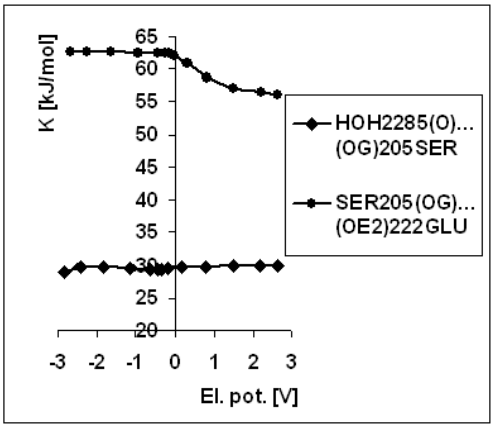

Fig. 4. Proton transfer parameter (K) vs. electrostatic potentials (El. pot.) curve of HOH2285(O)…(OG)205SER and SER205(OG)…(OE2)222GLU hydrogen bonds.

Figures 2-4 show that the electrostatic potential interval is between –3 to +3 [V]. The *K*-range is large (between 10^{-3} to 10^{2}). The reason of this phenomenon are donor-acceptor atom distances, which change between 2.59 Å to 2.79 Å. The value of K exponentially increases with donor-acceptor distance decrease. There are various shapes in Fig. 2-4 : linear, parabolic, S-shaped curves. The shapes are determine by the type of donor and acceptor atoms and the number of hydrogen bonds in which a single donor/acceptor atom forms (bifurcate hydrogen bonds). For example (OH)66GYS participate in three hydrogen bonds.

From bioelectronics point of view, the *K* vs. (El.pot.) characteristics of THR203(OG1) ….(OH)66GYS hydrogen bond is similar to reverse diode (with inverted *I-V* characteristic of a diode). The characteristic of the hydrogen bond formed by the chromophore and the water molecule GYS66(OH) is similar to the output characteristic of a filed effect transistor. On the other hand, there is no change observed in the hydrogen bond between HOH2285(O)….(OG)205SER when varying (El.pot.) in the *K* vs. (El.pot.) characteristics; hence, this hydrogen bond behaves similarly to a current source. Similar characteristics are obtained for hydrogen bonding network from β-lactamase protein [8].

The characteristics of the other hydrogen bonds – parabolic and S-shaped – no direct analogy to conventional microelectronic devices can be found. The characteristics are non-linear which implies amplifying properties.

In general, the hydrogen bonds might be compared to microelectronic devices; the proton donor and proton acceptor can be presented as drain and source electrodes. The sum of electrostatic potential (function of pH) in a given hydrogen bond can be presented as a gate electrode. In the investigations of the hydrogen bonding network, the chromophore atom GYS66(OH) can sum signals from different donors similar to microelectronic multiplexer. The hypothesis applies to GLU222 residue. The oxygen atoms OE1 and OE2 have strong proton acceptor properties. They take part in two separate hydrogen bonds. But due to the redistribution of electronic density between the two atoms, the proton transfers in the two bonds will influence onto each other.

If we consider the entire hydrogen bonding network as a single device, we find that the characteristics of its inputs at the acceptor GYS66(OH) are mirrored to the characteristics of its output at the donor GYS66(OG1) (cf. Fig. 2); both characteristics are parabolic, mirrored to each other with inflex point at approx. –0.5 V. Hence, the entire HBN will behave as an inverter.

IV. CONCLUSION

The study of proton transfer in hydrogen bonds with the chromophore of wild type green fluorescent protein shows that the proton transfer parameter depends on donor and acceptor electrostatic potentials, and the sum of protein electrostatic potential. The obtained curves of proton transfer parameters versus donor and acceptor electrostatic potentials are similar to *I-V* characteristics of 2- or 3-terminal devices. In addition, some of the characteristics are similar to the *I-V* characteristics of a reverse diode (a diode with an inverted characteristic), field effect transistor, current source. The chromophore and glutamine acid residues in the hydrogen bonding networks exhibit functions similar to microelectronic multiplexers.

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