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Analysis of Cis-Trans Photoisomerization Mechanism of Rhodopsin Based on the Tertiary Structure of Rhodopsin

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We propose a novel mechanism (Twist Sharing Mechanism) for the *cis-trans* photoisomerization of rhodopsin, based on the molecular dynamics (MD) simulation study. New things devised in our simulations are (1) the adoption of Mt. Fuji potentials in the excited state for twisting of the three bonds C9=C10, C11=C12 and C13=14 which are modeled using the detailed ab initio quantum chemical calculations and (2) to use the rhodopsin structure which was resolved recently by the X-ray crystallographic study. As a result, we found the followings: Due to the intramolecular steric hindrance between 20-methyl and 10-H in the retinal chromophore, the C12-C13 and C10-C11 bonds are considerably twisted counterclockwise in rhodopsin, allowing only counterclockwise rotation of the C11=C12 in the excited state. The movement of 19-methyl in rhodopsin is blocked by the surrounding three amino acids, Thr 118, Met 207 and Tyr 268, prohibiting the rotation of C9=C10. As a result only all-*trans* form of the chromophore is obtainable as a photoproduct. At the 90° twisting of C11=C12 in the course of photoisomerization, twisting energies of the other bonds amount to about 20 kcal/mol. If the transition state for the thermal isomerization is assumed to be similar to this structure, the activation energy for the thermal isomerization around C11=C12 in rhodopsin is elevated by about 20 kcal/mol and the thermal isomerization rate is decelerated by 10^{-14} times than that of the retinal chromophore in solution, protecting photosignal from the thermal noise.

Key words: Rhodopsin, *cis-trans* photoisomerization, MD simulation, one-way rotation, bond-specific isomerization

INTRODUCTION

The *cis-trans* photoisomerization of retinal chromophore of rhodopsin is the primary process of the visual excitation. This visual pigment has the following two important properties. The first is the high photosensitivity of rhodopsin. The isomerzation time is an order of 10^{-13}s. The thermal isomerization time is an order of 10^{11}s. The ratio is 10^{-23}. This very small ratio is inevitable so that one or two photons are detected by depressing the thermal noise for the visual excitation. The second is the high specificity of the *cis-trans* photoisomerization. Namely only the 11-*cis* to all-*trans* isomerization takes place.

So far much experimental and theoretical studies have been done in order to resolve this mechanism. However, satisfactory answer was not obtained so far. Under such situation, recently two important advancements were made. One is the clarification of the three dimensional structure of rhodopsin by the X-ray crystallographic study [1]. The other important advancement is the finding of the conical intersection (CI) point between the ground and excited state of the analog of protonated retinal Schiff-base by the much advanced quantum chemical calculations [2]. In this paper, making use of these achievements, we conduct the MD simulation for the *cis-trans* photoisomerization of rhodopsin.

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MATERIALS AND METHOD

In Figure 1a, we show a schematic picture of the potential energy surface (PES) as a function of minimum energy potential (MEP) coordinate calculated by the CASSCF method [2]. Using this PES, the photoisomerization takes place smoothly. Immediately after excited

to the FC state, bond length relaxation happens. After passing rather flat potential region, the system goes into a steep descent region and passes through the CI and reaches a photoproduct. Modeling this PES, we adopt such PES as a function of the twisting angle of the double bond as Figure 1b. We see a flat potential until ±60° twisting in the S_1 state and then sharp decrease follows to reach a CI point. From its shape we call Figure 1b Mt. Fuji potential. We adopt such Mt. Fuji potential for the three double bonds C9=C10, C11=C12 and C13=C14. Except the Mt. Fuji potential, we adopt the AMBER 4.1 all-atom force field. The MD simulation was made using program package PRESTO.

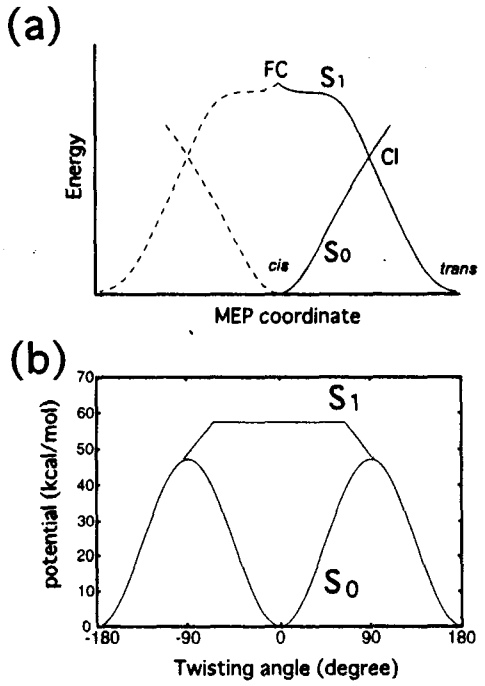

Figure 1: a) Schematic PES as a function of MEP for the *cis-trans* photoisomerization of the Protonated Schiff base of retinal. b) Mt. Fuji potential as a function of the twisting angle.

RESULTS

In Figure 2, the molecular structure of the retinal chromophore of rhodopsin is shown. It is the 11-*cis* form, having a steric hindrance between 20-methyl and 10-H. In solution, C12-C13 and C6-C7 are twisted by about 40° and 60°, respectively, and the other bonds in the conjugated region are not twisted.

][h]

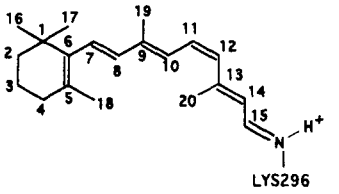

Figure 2: Molecular structure of 11-*cis* protonated Schiff-base of retinal.

In Figure 3, we show the calculated result of the average of 10 simulations for the twisting angles of the chromophore in rhodopsin before and after photoabsorption (at t=0, photon is absorbed).

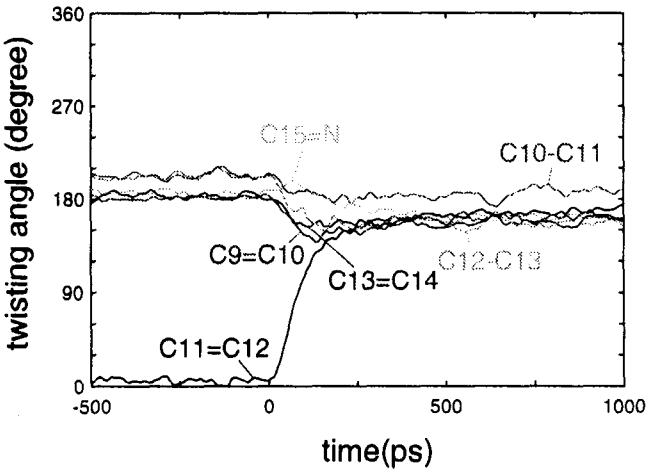

Figure 3: Simulated results of the twisting angle for some bonds of retinal chromophore as a function of time (at t=0, photoexcitation is made).

Before photoexcitation, C10-C11 and C12-13 single bonds are twisted counterclockwise by about 20°. The twisting of C10-C11 is brought about by the steric interaction of the chromophore with the protein environment. The smaller twisting of C12=C13 is due to the twisting of C10-C11, reducing the steric hindrance between 20-methyl and 10-H. Under such situation, there is no way other than the C11=C12 bond is twisted counterclockwise when photoexcited. Indeed, after photo-excitation, C11=C12 is rotated very quickly counterclockwise in about 160 fs. The other bonds C9=C10, C10-C11, C12-C13, and C13=C14 are twisted clockwise considerably. The bond C15=N is initially twisted counterclockwise

and later twisted clockwise. All these twistings take place in cooperation with the rotation of C11=C12. We call this cooperative mechanism Twist-Sharing model. In such a way we confirmed that the bond-specific, one-way *cis-trans* photoisomerization is attained in rhodopsin.

We made analysis which amino acids work to perform the bond-specific, one-way *cis-trans* photoisomerization of the chromophore. For this purpose, we made analysis of the intrinsic conformation change of the chromophore by the method developed by the author [3]. We make two parallel MD simulations by keeping the chromophore in the S_0 state all the time. In the other simulation, on the way of the MD simulation in the S_0 state, we suddenly change the chromophore into the S_1 state at certain time and continue the MD simulation in the S_1 state. Then, we calculate the difference of the atom position $|\mathbf{r}(t, S_1) - \mathbf{r}(t, S_0)|$. In Figure 4, we show the simulation result obtained by ten runs for the intrinsic displacement of each atom of rhodopsin between t=0 and 130 fs. The largest displacement are those of the H11 and H12 of the chromophore. We also see that the displacement of water atom is large.

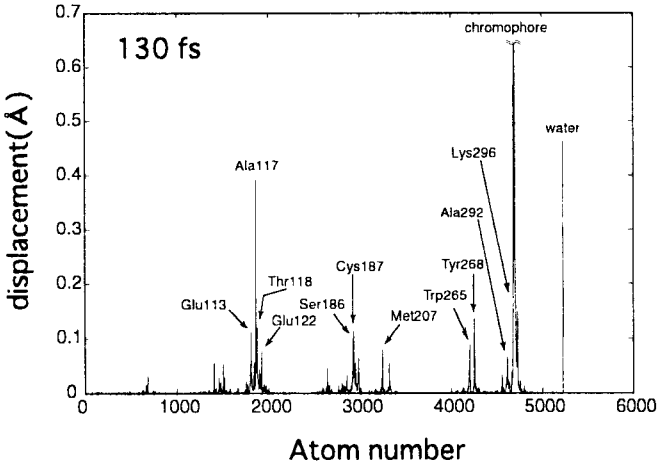

Figure 4: Intrinsic conformation change for sequentially numbered atom in rhodopsin calculated by $|\mathbf{r}(t, S_1) - \mathbf{r}(t, S_0)|$ at 130 fs.

The amino acids with large displacement were Ala117, Lys296, Ala292, Tyr 268, Trp265, Met 207, Cys187, Ser186, Glu122, Thr118 and Glu113. Drawing the space filling model of rhodopsin, we found that the above amino acids form the binding pocket of the chromophore. The amino acid Ala117 which had the largest value of the displacement among the amino acids just contacts with C12 of the chromophore. We also see that 19-methyl of the chromophore is tightly surrounded by Thr118, Met207 and Tyr268.

Since 19-methyl of the chromophore is tightly surrounded by the three amino acids, the movement of the 19-methyl might be blocked during the *cis-trans* photoisomerization. This will be the reason why C9=C10 bond is not rotated simultaneously with C11=C12 rotation. If 19-methyl is substituted by a small atom H, the steric restriction would be much reduced and another channel for the *cis-trans* photoisomerization must be open. Then, we did *cis-trans* photoisomerization simulation using 9-demethyl rhodopsin (19-methyl is substituted with H). We found that 9-*cis* form of the photoproduct is produced with the yield of 90% by the bicycle pedal mechanism. Then, the role of 19-methyl is very important to close the channel of the *cis-trans* photoisomerization to yield the 9-*cis* form of the photoproduct. We did the other modification to produce mutant rhodopsin where Thr118 is substituted by Gly. Since the size of Gly is much smaller than Thr, the ability of the confinement of the 19 methyl of Gly is expected to be much reduced as compared with Thr. The simulation result for this mutant is that partially 9-*cis* form of the photoproduct is produced. In such a way, the block of the movement of 19-methyl of the chromophore by the three amino acids Thr118, Met207 and Tyr268 is essential to lead to only all-*trans* form of the photoproduct.

The above results are summarized in Table 1.

We also did MD simulations for 13-demethyl rhodopsin (20-methyl is substituted with H) where the steric hindrance between 20-methyl and 10H is deleted. Therefore, there is no restriction for the C11=C12 bond to rotate clockwise. The result is that many kinds of photoproduct including 9-*cis*, 13-*cis* form as well as all-*trans* form are produced. From this result, we find that the intramolecular steric hindrance between 20-methyl and 10H is essential to prohibit the production other than all-*trans* form of the chromophore.

DISCUSSION

On the basis of the above results, we may summarize the origin of high specificity of photoisomerization as follows: Due to the intramolecular steric hindrance between 20-methyl and 10H, the C12-C13 and C10-C11 single bonds are twisted counterclockwise in rhodopsin, allowing only counterclockwise rotation of C11=C12 in the *cis-trans* photoisomerization. The movement of 19-methyl in rhodopsin is blocked by the surrounding three amino acids, Thr118, Met207 and Tyr268, prohibiting

Table 1: Photoproducts of native and modified rhodopsins.

case	rhodopsins	photoproducts	ratio of products
1	Native rhodopsin	all-*trans* (11=12:CC)	10/10 (100%)
2	9-demethyl rhodopsin	all-*trans* (11=12:CC)	1/10 (10%)
		9-*cis* (11=12:CC, 9=10:C)	9/10 (90%)
3	T118G rhodopsin	all-*trans* (11=12:CC)	7/10 (70%)
		9-*cis* (11=12:CC, 9=10:C)	3/10 (30%)

CC indicates counterclockwise rotation. C indicates clockwise rotation.

the rotation of C9=C10. Only all-*trans* form of the chromophore is obtainable as a product of photoisomerization. When Thr118 is substituted by Gly, the clockwise rotation of C9=C10 becomes to be allowed, as well as the simultaneous counterclockwise rotation of C11=C12, giving rise to 9-*cis* form of the chromophore as a photoproduct.

We may summarize the origin of high photosensitivity of rhodopsin as follows. At the 90° twisting of C11=C12 in the course of photoisomerization, twisting energies of the other bonds amount to about 20 kcal/mol. If the transition state for the thermal isomerization is assumed to be similar to this structure, the activation energy for the thermal isomerization around C11=C12 in rhodopsin is elevated by about 20 kcal/mol and the thermal isomerization rate is decelerated by 10^{-14} times than that of the retinal chromophore in solution (ca. 100 s^{-1}). The *cis-trans* photoisomerization in rhodopsin takes place ultrafast in about 160 fs. Combining the above properties, extremely high sensitivity (10^{-25}) of photoreception is realized.

CONCLUSION

We analyzed an important role of protein environment in the *cis-trans* photoisomerization of rhodopsin, based on the MD simulation study. For this purpose, we adopted Mt. Fuji shaped model potential surface for twistings of the three double bonds C9=C10, C11=C12 and C13=C14 in the excited state of retinal chromophore. We obtained a result that only the C11=C12 bond in the *cis* form of the chromophore is rotated counterclockwise to yield all-*trans* form of the chromophore by the photo-excitation, and a rapid phase of the *cis-trans* photoisomerization is virtually completed in about 160 fs, in consistent with the experimental result. We found that many single and double bonds neighboring the C11=C12 bond are all twisted clockwise to a certain amount, in the course of realizing one-bond rotation around the C11=C12 bond in a tightly confined space of the binding site. We call this cooperative photoisomerization mechanism *Twist sharing one-bond rotation mechanism.* This *cis-trans* photoisomerization mechanism was analyzed in detail using a modified rhodopsin with 9-demethyl retinal as a chromophore and T118G rhodopsin mutant (Thr118 being substituted by Gly). We found that Thr118 plays a key role in blocking the movement of 19 methyl of the chromophore, prohibiting the formation of 9-*cis* form as a photoproduct by the bicycle pedal mechanism.

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