Paper

Spacing Between Retinal and Amino Acid Residues in Squid Rhodopsin

Yoshinori Nagai[1]*, Masashi Kito[1] and Ted Maddess[2]

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Abstract: In order to understand the fundamental basis of polarized light recognition, the distance between selected atom pairs is calculated using crystallography data found in PDB data of squid rhodopsin. We quantify the location site of retinal and distance change between retinal and amino acid residues of squid rhodopsin. Based on this evidence we speculate on rhodopsin being a molecular machine or molecular engine driven by photo absorptions.

1. Introduction

The retinal houses the first step of light absorption in rhodopsin. It is also the first step of visual system. Our previous work considered the dipole moment of retinal and huristic molecular orbital calculations [1]. Here we investigate how retinal is located within rhodopsin. We found two new crystallography data sets in the Protein Data Bank (PDB) [2], with IDs 3AYM and 3AYN. Altogether we use four PDB data set for squid rhodopsin (2Z73 [3], 2ZIY [4], 3AYM [5], 3AYN [5]) in the present study. We also use the chain B structure of the PDB data to compare conformations of retinal and protein chains between PDB data. PDB 2Z73, 3AYM, and 3AYN have two chains A and B, whereas 2ZIY has only an A chain. It is stated in the PDB that titles of 2Z73, 2ZIY and 3AYN are the same, namely the crystal structure of squid rhodopsin and title of 3AYM is the crystal structure of the batho-intermediate of squid rhodopsin. The length of the amino acid sequences and the crystallized regions are summarized from the PDB data [2]. Those results are presented in Table I.

Table I Length of residues and crystallized region of chains A and B

PDB	Length	Chain	Crystallized region
2Z73	448	A	9–358
		B	9–355
2ZIY	372	A	4–373
3AYM	448	A	9–358
		B	9–355
3AYN	448	A	9–358
		B	9–355

* Correspondence author
[1] Center for Information Science, Koksuhikan University, Tokyo
[2] Joh Curtin School of Medical Research, ARC Centre of Excellence in Vision Science, Australian National University, Canberra

The amino acid sequences for these PDB data of squid rhodopsin are identical to each other. The only difference within the data comes from a few missing amino acid residues and uncrystallized parts, such that those four kinds of PDB data are highly comparable. We therefore use these PDB data for analysis of retinal location sites and the spacing between retinal and the opsin amino acid residues. The analysis procedure is to calculate the distance between pairs of selected atoms. We present the results both in graphical form and descriptions in the text of Section 2 and 3.

In Section 2, we show the location site of retinal within the squid rhodopsin, using contact maps or distance maps of the alpha carbons of the rhodopsin protein, and distance maps between retinal carbons and alpha carbons of the amino acid residue of squid rhodopsin. This distance becomes larger than the distance between retinal carbons and atoms of amino acid residue allowing us to find the nearest atom in the rhodopsin protein from every retinal carbon atom. Those results are shown in Section 3. Considering those results we speculate in Section 4 on what happens in rhodopsin with ligated retinal after photon absorption has occurred from the view point of a molecular machine or molecular engine. Section 4 also includes summary and discussion.

2. Location site of retinal in a squid rhodopsin

To understand the interaction between the retinal and the opsin protein, the carbon numbering of retinal was investigated and the result is shown in Fig. 1. The location of retinal within the rhodopsin protein refers to which amino acid residue has the shortest distance from every retinal carbon. We should also investigate what kind of structure the rhodopsin protein has. For this purpose, we used contact maps or distance maps, either between pairs of amino acid residues, or between retinal carbons and amino acid residues.

Distance maps between all pairs of residues within rhodopsin were investigated for 2Z73 chain A and B, 2ZIY chain A, 3AYM chain A and B, and 3AYN chain A and B. Distance maps less than 1.5 nm were made for the available A and B chain data sets. The results are quite similar to each other for all chains, so that we only show in Figs 2A and 2B the distance maps or contact maps for 2Z73A and 2Z73B, respectively. Here we abbreviate 2Z73 chain A as 2Z73A. In this calculation we utilized alpha carbons (C_α).

Figs 3A and 3B denote distances between retinal carbons and the C_α of residues in the rhodopsin. The vertical direction of Figs 3A and 3B denotes retinal carbons, and the horizontal direction of these figures the number of amino acid residues. The coordinate used was C_α of each residue.

As seen from Figs 3A and 3B, it is clear that the B chains of squid rhodopsin have a compact

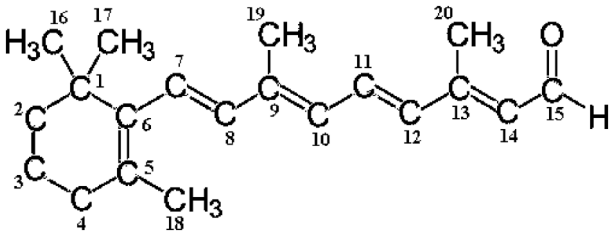

Fig. 1 Carbon numbering of retinal

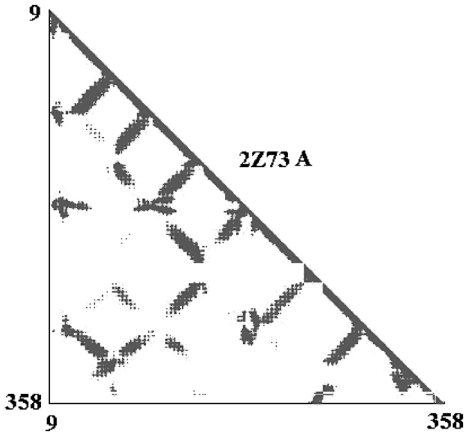

Fig. 2A An example of Distance map of Chain A. Plots less than 1.5 nm.

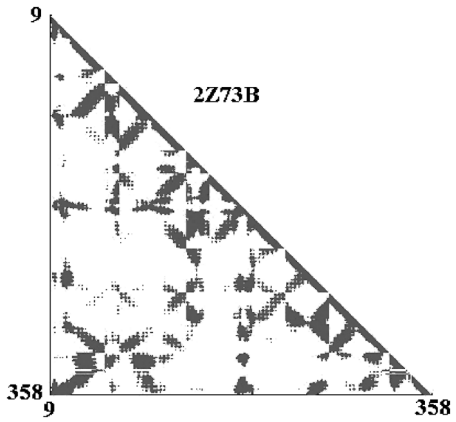

Fig. 2B An example of Distance map of Chain B. Plots less than 1.5 nm.

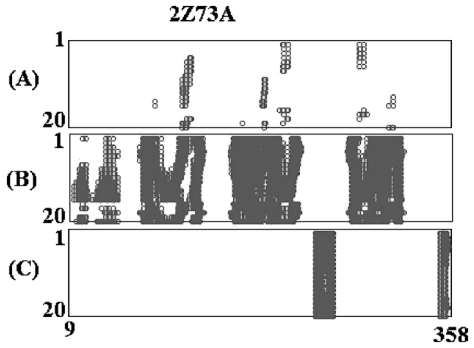

Fig. 3A Distance between amino acid residues and retinal carbons of chain A. The horizontal axis denotes residue number and the vertical axis the retinal carbon number.
(A): Distance less than 0.7 nm.
(B): Distance between 1.0 nm and 2.0 nm.
(C): Distance greater than 5.0 nm.

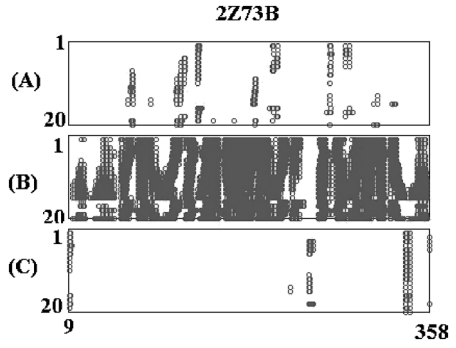

Fig. 3B Distance between amino acid residues and retinal carbons of chain B. The horizontal axis denotes residue number and the vertical axis the retinal carbon number.
(A): Distance less than 0.7 nm.
(B): Distance between 1.0 nm and 2.0 nm.
(C): Distance greater than 3.0 nm.

shape close to the retinal. We couldn't find any C_α that was located at a distance greater than 4 nm from the retinal carbons. Row (B) of Figs 3A and 3B clearly shows that number of residues in the range of distances between 1 nm and 2 nm relative to chain B becomes high in comparison to chain A. This fact means that the distance between retinal carbons and amino acid residues of squid rhodopsin is shorter in the chain B. This fact also is confirmed by distance maps of the rhodopsin C_α sequence as shown in Figs 2A and 2B. The contact area of 2Z73A decreased relative to that of 2Z73B. As the result, it can be imagined that changes in the rhodopsin volume occur with the conformation changes of retinal.

Table II Nearest atom of the residue to retinal carbons for chain A

PDB	C_1	C_2	C_3	C_4	C_5	C_6	C_7
2Z73A	Phe205CZ	Ala278CB	Trp274CB	Phe209CD1	Trp274CE3	Phe120CE2	Phe120CE2
2ZIYA	Phe120CE1	Phe209CB	Phe209CD1	Trp274CB	Trp274CE3	Phe120CE1	Phe120CZ
3AYMA	Phe209CB	Phe209CD2	Phe209CG	Phe209CD1	Phe120CE2	Phe120CE2	Phe120CE2
3AYNA	Phe205CE1	Phe209CD2	Trp274CB	Trp274CE3	Trp274CE3	Phe120CE2	Phe120CE2

PDB	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}
2Z73A	Phe188CE2	Gly116CA	Gly116CA	Gly112O	Ser187OG	Lys305NZ	Lys305NZ
2ZIYA	Phe188CD1	Phe188CD1	Phe188CD1	Gly112O	Se187CA	Lys305NZ	Lys305NZ
3AYMA	Trp274CZ3	Gly116CA	Trp274CZ3	Gly116N	Trp274CH2	Lys305NZ	Lys305NZ
3AYNA	Phe188CE2	Gly116CA	Gly116CA	Ser187OG	Ser187OG	Ser187OG	Lys305NZ

PDB	C_{15}	C_{16}	C_{17}	C_{18}	C_{19}	C_{20}
2Z73A	Lys305NZ	Phe205CD2	Phe205CZ	Gly119C	Gly116CA	Trp274CZ3
2ZIYA	Lys305NZ	Phe120CE1	Phe205CE1	Trp274CE3	Gly116CA	Trp274CZ3
3AYMA	Lys305NZ	Phe205CA	Phe205CZ	Gly119C	Phe118CD2	Ser187OG
3AYNA	Lys305NZ	Phe205CG	Phe188CE2	Trp274CZ3	Met204SD	Trp274CZ3

Table III Nearest atom of the residue to retinal carbons for chain B
Note that 2ZIY has no chain B data.

PDB	C_1	C_2	C_3	C_4	C_5	C_6	C_7
2Z73B	Arg133CB	Arg133CB	Ser263OG	Ser263OG	Ile129O	Ile129O	Ile129O
2ZIYB							
3AYMB	Arg133CB	Arg133CB	Arg133CG	Ile129O	Ile129O	Ile129O	Ile129O
3AYNB	Arg133CB	Arg133CB	Ser263OG	Ser263OG	Ile129O	Ile129O	Ile129O

PDB	C8	C9	C10	C11	C12	C13	C14
2Z73B	Arg133NH1	Ala69CB	Ala69CB	Asn70ND2	Asn70ND2	Asn70ND2	Asn70ND2
2ZIYB							
3AYMB	Ile129O	Ala69CB	Trp274CH2	Asn70ND2	Asn70ND2	Asn70ND2	His319NE2
3AYNB	Asp132OD1	Ala69CB	Ala69CB	Asn70ND2	Asn70ND2	Asn70ND2	Lys305NZ

PDB	C_{15}	C_{16}	C_{17}	C_{18}	C_{19}	C_{20}
2Z73B	Lys305NZ	Arg133N	Arg133CD	Ile129O	Ala69CB	Leu259CD1
2ZIYB						
3AYMB	His319NE2	Arg133N	Arg133CD	Ile129O	Asp132OD1	Ser187CB
3AYNB	Lys305NZ	Arg133N	Arg133CD	Ile129O	Ala69CB	Leu259CD1

3. Nearest atom of the amino acid residue from every retinal carbon

Since the sizes of amino acids differ, amino acids with longer side chains are closer to the retinal carbons compared to the distance of small amino acids. We therefore investigated the nearest atom of each residue for every retinal carbon. The data obtained are tabulated in the previous page.

We also calculated the distance, in Angstrom units, from each retinal carbon to the nearest atom of the rhodopsin residues. Figs 4A and 4B show these values. On average chain B displayed 0.05 nm shorter distances compared to chain A. The greatest shortenings occurred at C_1, C_2, C_6, C_{12}, C_{16}, C_{17}, and C_{19}. Except for C_{19} these carbons in retinal are next to a 6 member ring and a side

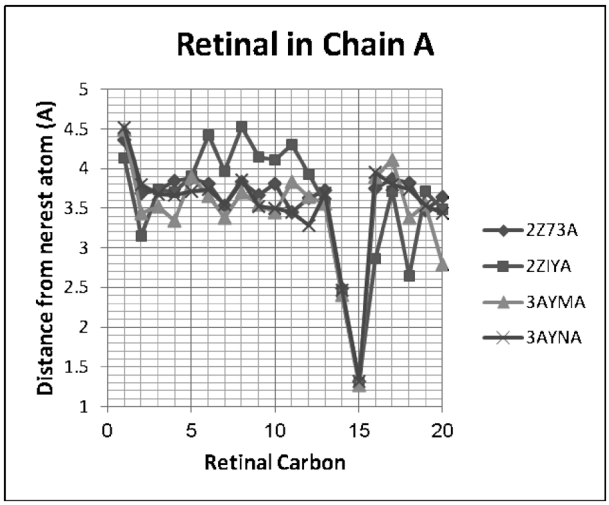

Fig. 4A Distance between retinal carbons and the nearest atom of amino acid residues in chain A.

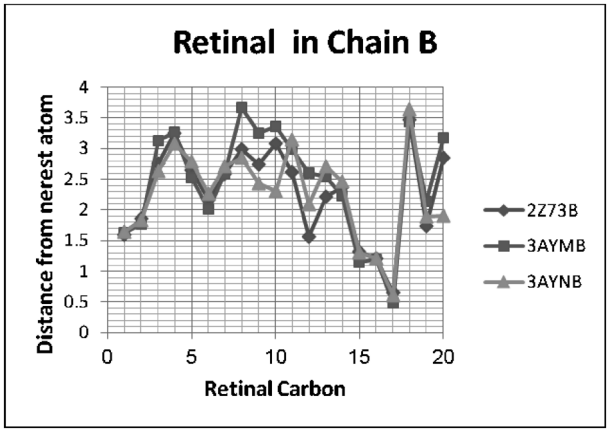

Fig. 4B Distance between retinal carbons and the nearest atom of amino acid residues in chain B.

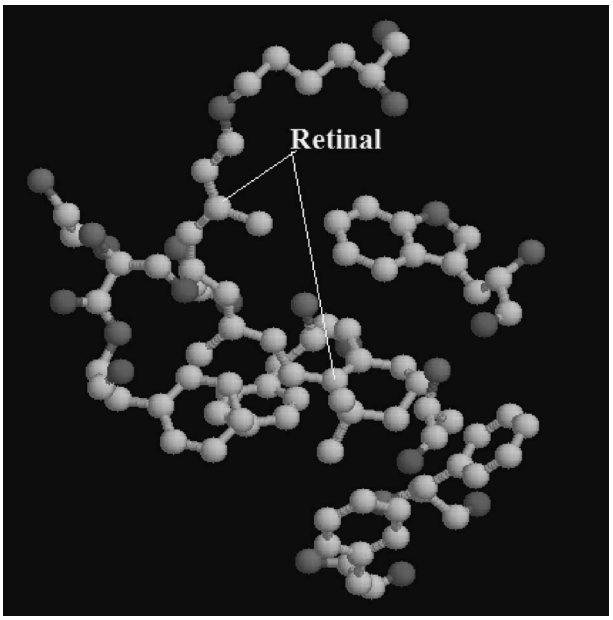

Fig. 5A Retinal and contact amino acid residues in chain A.

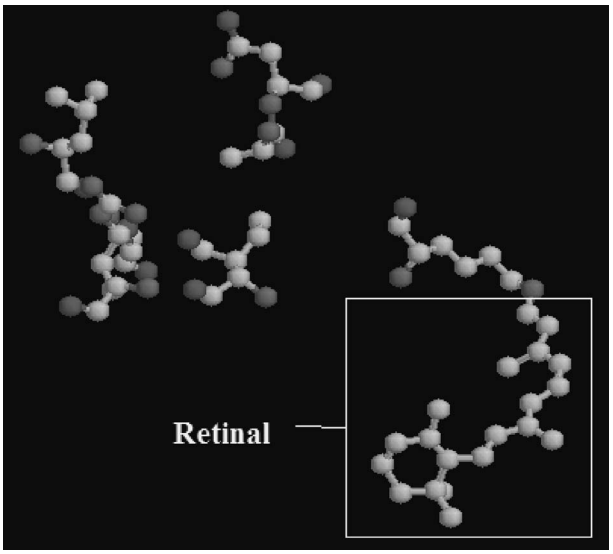

Fig. 5B Retinal and contact amino acid residues in chain B.

group binding to C_1. Carbon C_{19} is bound to C_9 and is located near C_{10} and C_{11} (Fig 1) implying that there are related to the transition change between Cis- and Trans-conformations of retinal, namely, tightly linked to the phenomena of photon absorption by retinal [1]. We also note that the nearest atom distance for C_{15} shows little different in the two chains. This implies C_{15} might be bound to Lys 305 (except for 3AYNB).

In order to see how amino acids contact to retinal, we used the molecular viewing software RasMol to draw the molecules. Figs 5A and 5B show contact residues and retinal in chain A and chain B respectively.

The distances between amino acids and retinal carbons in Fig. 5A are longer than those in Fig. 5B. But the present images give us opositte feeling. It should be considered as follows, the retinal in Fig. 5B denotes that only C_{15} binds to amino acid residues. In Fig. 5A, the six member ring of retinal has much interaction with amino acid residues of rhodopsin. This implies that the retinal is spread out within rhodopsin molecules. Actually chain A monomer is spread more than the chain B monomer in squid rhodopsin of PDB data.

4. Summary and Conjecture

A distance analysis of chains A and B of squid rhodopsin crystallography data obtained from PDB shows that B chains are more compact than A chains. Alternatively we can say that the monomer of chain A is more spread out than the monomer of chain B. It is a well known fact that retinal absorbes photons, i.e. that retinal obtains energy from light. From a thermodynamical viewpoint [6], photon absorbtion makes thermal work. As was shown by the evidence obtained from distance analysis, volume change will occurr in rhodopsin molecules. If the retinal included in chain B takes a closer form to the cis-conformation than retinal included in chain A, a photon absorbed by retinal makes the conformation change to trans. The rhodopsin molecule does work via photon absorption. In other words rhodopsin is a transducer. Moreover, introducing retinal states for A and B in rhodopsin, we can imagine a cycle of going and back and forth between states A and B. Indeed in cephalopods and some other invertebrates (but not vertebrates) the absorption of different wavelengths of light reverses the cis to trans conformal change associated with light perception [7, 8]. This suggests a molecular engine driven by photon energy. In this sense we can imagine rhodopsin as a kind of molecular machine.

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