Unique Mechanisms of Excitation Energy Transfer, Electron Transfer and Photoisomerization in Biological Systems

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Abstract. We discuss unique mechanisms typical in the elementary processes of biological functions. We focus on three topics. Excitation energy transfer in the light-harvesting antenna systems of photosynthetic bacteria is unique in its structure and the energy transfer mechanism. In the case of LH2 of *Rhodospseudomonas acidophila*, the B850 intra-ring energy transfer and the inter-ring energy transfer between B800 and B850 take place by the intermediate coupling mechanism of energy transfer. The excitonic coherent domain shows a wave-like movement along the ring, and this property is expected to play a significant role in the inter-ring energy transfer between LH2's. The electron transfer in biological systems is mostly long-range electron transfer that occurs by the electron tunneling through the protein media. There is a long-standing problem that which part of protein media is used for the electron tunneling root. As a result of our detailed analysis, we found that the global electron tunneling root is a little winded with a width of a few angstrom, reflecting the property of tertiary and secondary structures of the protein and it is affected by the thermal fluctuation of protein structure. Photoisomerization of rhodopsin is very unique: The *cis-trans* photoisomerization of rhodopsin occurs only around the C11=C12 bond in the counterclockwise direction. Its molecular mechanism is resolved by our MD simulation study using the structure of rhodopsin which was recently obtained by the X-ray crystallographic analysis.

Key words: electron tunneling root, excitation energy transfer, exciton, long-range electron transfer, photoisomerization, retinal chromophore

1. Introduction

Light energy from the sun is a sole energy source of all the living things on the earth. The light energy is caught by antenna pigments (chlorophylls, carotenoides and so on) in photosynthetic bacteria, algae and plants. The absorbed energy by the electronic excitation is successively transferred among the pigments and finally trapped to the reaction center [1]. This transfer process is called excitation energy transfer or shortly energy transfer. The energy transfer is of fully quantum mechan-

ical phenomenon. The energy transfer under the thermal agitation of the electronic state through the electron-phonon interaction should display various aspects of the energy transfer, from the exciton mechanism [2, 3] through the intermediate coupling mechanism [4, 5] to Förster mechanism [2]. These are very interesting physical problems which are not solved yet in detail. Similarly, it is also an important task to examine what type of mechanism is used in each biological system for an efficient overall energy transfer.

The excitation energy delivered to the reaction center is used to induce charge separation reaction between special dimer and the primary acceptor, forming cation of dimer and anion of the acceptor. The electron on the anion acceptor is then successively transported to the secondary acceptor and so on. Finally, it is transported to quinone in purple bacteria, PSII and so on [6, 7]. Such transportation of electron is called electron transfer. The successive electron transfer is necessary for escaping from the non-productive charge recombination reaction. For this purpose, electron transfer rate at each step is regulated and the system is devised so that optimum yield of the reduced state of quinone is obtained. This long-range electron transfer takes place by the electron tunneling mechanism through protein media. Therefore, it is an important task to examine how protein media work to regulate the electron transfer root and then the electron transfer rate.

Light is also used in the visual transduction, or simply vision [8]. The light absorption is made by visual pigments. The visual pigment of photopic vision is rhodopsin. Rhodopsin involves a chromophore of 11-*cis* retinal forming a Schiff-base linkage with lysin of opsin. The light absorption by rhodopsin causes *cis-trans* photoisomerization of the chromophore in ultra-short time [9, 10]. On the other hand, the thermal isomerization of the chromophore takes a long time, ca. 10^{11} s [11]. The ratio of both isomerization times is as small as 10^{-24} , indicating quite high sensitivity of visual excitation. There is a rigid specificity that only the photoisomerization around the 11-*cis* bond takes place. The molecular mechanisms of the very high photosensitivity and high specificity are not solved yet.

In this paper, we present some new results of theoretical analysis of the above problems significant in photobiology and physics.

2. Excitation Energy Transfer

So far, two kinds of energy transfer mechanisms are well-known. One is the exciton mechanism which applies to the strong excitonic coupling case and has a character of coherent excitation energy transfer [2, 3]. The other is the Förster mechanism which applies to the weak excitonic coupling case and has a character of incoherent excitation energy transfer [2]. However, there must be the third mechanism of the energy transfer in the intermediate coupling case [4, 5]. This third mechanism is not well-investigated but is important in biological energy transfer, as we show below.

Recently we formulated the energy transfer rate for dimer which applies to all the excitonic coupling strengths, by self consistently solving the stochastic Liouville equation with a decoupling procedure which corresponds to the factorization by the two-time correlation functions [5]. From this result, we could derive the degree of coherence η using the power of U in the expression of energy transfer rate. We can show that when η is larger than a critical value $\eta_c (= 0.5286\dots)$, excitonic behavior remains while it does not in the opposite case. In order to extend this theory, we make use of the generalized master equation (GME) for the population P_a of the excitation of the chromophore at site a using a memory function which is obtained by the second order perturbation method as follows:

$$\frac{\partial P_a(t)}{\partial t} = \int_0^t ds \sum_b \{W_{ab}(t-s)P_b(s) - W_{ba}(t-s)P_a(s)\} \quad (1)$$

$$W_{ab}(t) = \begin{cases} \frac{2U_{ab}^2}{\hbar^2} \exp\left(-\frac{\gamma_{ab}}{\hbar}t\right) & \text{for } b \text{ neighbouring } a \\ 0 & \text{others} \end{cases} \quad (2)$$

$$\gamma_{ab} = \sqrt{\frac{4k_B T'(\lambda_a + \lambda_b)}{\pi}} \exp\left[\frac{(\Delta G + \lambda_a - \lambda_b)^2}{4k_B T'(\lambda_a + \lambda_b)}\right] \quad (3)$$

$$k_B T' = \frac{1}{2} \hbar \omega \coth\left(\frac{\hbar \omega}{2k_B T}\right). \quad (4)$$

Here, γ is the decay factor and works as destructing the coherence, $\hbar$ is the Planck constant divided by 2π , ΔG_{ab} is the energy gap of the chromophores at site a and b , λ_a and λ_b are the reorganization energies for optical absorption of the chromophores at site a and b , and T' is the effective temperature.

We solved the GME using parameter values of $U = 350 \text{ cm}^{-1}$, $\gamma = 140 \text{ cm}^{-1}$, $T = 300 \text{ K}$ and $\omega = 20 \text{ cm}^{-1}$, by exciting the end chromophore initially. We calculated the degree of coherence at each chromophore. We find that η becomes η_c for the chromophore separated from the end chromophore by 3.7. Then we assign the coherence length (number of the chromophores involved in the coherent domain) $N_{coh} = 4.7$. We do such calculations by varying the values of U and γ . As a result, we obtained the following empirical formula

$$N_{coh} = 1.41 + 1.32 \frac{U}{\gamma}. \quad (5)$$

The above empirical formula can be qualitatively interpreted as follows: Let us consider some part of the chromophore forms an excitonic coherent domain in the linear array of chromophores. In the equilibrium state, the coherent domain is moving and fluctuating in its length by the shrink and expansion due to the competition of formation and destruction forces of coherent bonds. Let us assume

that the creating force as an excitonic coupling strength U and the destructing force as the decay constant in the memory function γ . It should be remembered that γ is not the homogeneous broadening in the optical absorption spectrum of the chromophore but is an inverse of the Franck-Condon factor for the energy transfer between the neighboring chromophores as shown in Equation 3. The formation time of the coherent bond is about $\hbar/U$. The lifetime of the coherent bond is about $\hbar/\gamma$. Then, the coherent domain length in the equilibrium state, shortly coherent length N_{coh} , is approximately expressed as $U/\gamma + 1$. We find that this formula is quite similar to the empirical formula obtained above. Based on these facts, we can qualitatively assign that the creating force of the coherent bond is U and the destructing force of the coherent bond is γ . When N_{coh} is smaller than the length of the linear array of chromophores and larger than 1.5, we say that the intermediate coupling energy transfer mechanism applies.

Now we apply this method to the LH2 of photosynthetic bacteria *Rhodospseudomonas acidophila*. In this LH2, there are two kinds of rings composed of bacteriochlorophylls (BChl). One being closely packed by 18 BChls is called B850. The other being loosely packed by 9 BChls is called B800. We measured the coherence length in the equilibrium state of B850 and B800. The proper parameter values of U for B850 and B800 are 350 cm^{-1} and 25 cm^{-1} , respectively [1]. The value of γ is chosen as 140 cm^{-1} using the reorganization energy 40 cm^{-1} of single, BChl [12] for both of B850 and B870. Then, we obtained a result that N_{coh} is 4.7 for B850 and 1.6 for B800. Namely, the intermediate coupling energy transfer mechanism applies for B850. The mechanism of the energy transfer in B800 is critical between the intermediate coupling case and weak coupling case.

Assuming that the excitation energy is initially localized at one BChl in B850, we solved GME to see how the energy transfer takes place from B800 to B850. The result is shown in Figure 1. Top is the graph of excitation population remaining at B800 as a function of time. We see rather dull decrease of the population in the early time region, representing a trace of coherent character of the energy transfer. For comparison, we plotted the result calculated by solving the Pauli Master equation (PME) by thin line. Bottom is the plot of the population at each BChl in B850 as a function of time. We see that the excitation energy is transferred from B850 to the nearest BChl in B850 and then it is transferred successively. Remarkable feature is that some wavy part is added to the increasing curve of the population and time delay exists for the onset of the increasing curve at BChls far apart from the initially excited BChl. These anomalous behaviors are not seen in the curves obtained by PME which are represented by the thin lines. These two remarkable phenomena obtained by solving the GME are representative of the coherent character in the intermediate coupling case. Therefore, it is not necessarily a good approximation to assume that the exciton state exists over the whole ring of B850 from the first.

Figure 1. Time course of the populations for the energy transfer from B800 to B850 solved by GME (bold line) and PME (thin line). Top: Time course of the population at the BChl initially excited in the B800 ring. Bottom: Time course of the population at each BChl in the B850 ring.

3. Electron Transfer in Protein Media

The electron transfer (ET) in biological systems takes place mostly by the electron tunneling through protein media. In such case, the superexchange mechanism works. Namely, the electronic wavefunction of donor extends to the acceptor site by mixing the electronic wavefunctions of media. The electronic wavefunction of acceptor extends to the donor site by mixing the electronic wavefunctions of media. As a result, overlap of the effective electronic wavefunctions between donor and acceptor is enhanced very much. Two conditions are required due to the tunneling mechanism. One is that the overlap of the electronic wavefunctions between initial and final state must be large. The other is that the energy must be conserved before and after electron tunneling. Then, the electron transfer rate k_{ET} is written as follows:

$$k_{ET} = \frac{2\pi}{\hbar} |T_{DA}|^2 (FC) \quad (6)$$

where T_{DA} is the electronic factor corresponding to the overlap of the electronic wavefunctions between initial and final states. (FC) is the nuclear factor (Franck-Condon factor) justifying the energy conservation condition. This factor gives rise to the Marcus energy gap law [13, 14]. In the following, we focus on the electronic factor.

One of the most interesting problems in the electronic factor is through what root the electron tunneling takes place in protein media. There were two kinds of typical models. One is the pathway model proposed by Beratan and Onuchic [15–19]. They considered that the tunneling electron is most likely to pass through chemical bonds, next likely through hydrogen bonds and least likely through spaces. The possible pathway is guessed by choosing the atom-sequential roots connecting donor and acceptor involving hydrogen bonds and spaces as least as possible. This model was applied to myoglobin derivatives, azurin derivatives and cytochrome derivatives [20–24]. They found that the ET rate has better correlation with the pathway length than the donor-acceptor length. So, they emphasize that the secondary and tertiary structures of protein should regulate the electron tunneling root. This model was later improved so that some small atomic groups are incorporated in the chemical bond, called tube model [25, 26].

The other typical model is called Dutton plot [27, 28]. They plotted logarithm of the ET rate as a function of donor-acceptor distance for various redox pairs mostly taken from photosynthetic systems. They found that a linearly decreasing line with a slope $\beta = 1.4 \text{ \AA}^{-1}$ fits those data considerably well. They also showed that if the atomic packing density of the protein media involved in the tube connecting donor and acceptor is taken into account in the slope, the above correlation became much better. Therefore, it gives a picture that the ET rate is essentially determined by the donor-acceptor distance and the slope β is somewhat modified by the atomic packing density of the media on the way of connecting donor and acceptor. This picture is different from that of the pathway model where the tunneling root and the

ET rate are expected to be affected greatly by the secondary and tertiary structures of protein.

Recently, we proposed the third model, called worm model [29], which consolidates the above different pictures of the two typical models. We calculated all the inter-atomic tunneling currents of protein by the extended Hückel MO method [30]. As a typical system, we choose Ruthenium-modified azurin. Donor is Cu^+ which is involved in natural azurin. Acceptor is Ru^{3+} complex ligating to His residue which is inserted at any place of protein by the site-directed mutagenesis. These experimental systems were invented by Gray *et al.* [20–24]. The distribution of the calculated inter-atomic tunneling currents spreads considerably and it is similar to the distribution of the pathway model. In this sense, our model expressed by the tunneling current [30] is consistent with the pathway model. Next, we take statistical average of those tunneling currents. For this purpose, we put planes perpendicularly to the line connecting donor and acceptor. We make mark for the crossing points of inter-atomic tunneling currents to each plane. We put a weight of current amplitude for each crossing point. Then, we take statistical average for those points, obtaining the barycenter and standard deviation in each plane. Then, we write the circle with radius of the standard deviation centered at barycenter in each plane. Connecting those circles successively, we obtain the effective tunneling region. The numerical results showed that this tunneling region is a little winded with a width of 2.5–3.0 Å in diameter. From its shape, this tunneling region is called worm [29]. The worm is not so much different from the straight pipe connecting donor and acceptor, indicating the similar picture proposed by Dutton *et al.* [27, 28]. Therefore, the worm model based on the inter-atomic tunneling current consolidates the pathway model with the Dutton plot. Namely, the picture of pathway model is obtained when we look at the electron tunneling pathway microscopically. The picture of the Dutton plot is obtained if we look at the electron tunneling phenomenon globally by means of the statistical average.

Another important problem is how much the electronic factor can be affected by the thermal fluctuation of protein conformation. Non-negligible effect of thermal fluctuation was reported already [31, 32]. In order to examine this, we conducted MD simulation for 10 ps after attaining the thermal equilibrium. Then, we calculated electronic factor $|T_{DA}|$ for each configuration of the Ruthenium-modified azurin during 10 ps. We calculated 6 kinds of acceptors which are Ru^{3+} complex ligated to His residues produced at 6 different positions. The calculated results of the electronic factor $|T_{DA}|$ as a function of the donor-acceptor distance is shown in Figure 2. We see that the fluctuation of $|T_{DA}|$ amounts to about 2 orders of magnitude for all the acceptors. We also see some fluctuation for the donor-acceptor distance but it is not large enough to explain the observed fluctuation of $|T_{DA}|$. We analyzed the origin of the fluctuation of $|T_{DA}|$ based on the distribution of inter-atomic currents. We found that there are two kinds of conformations. One is the conformation with destructive interference where the absolute values of tunneling currents do not change but the sign of the currents varies considerably in

Figure 2. The effect of thermal fluctuation of protein conformation on the electronic factor $|T_{DA}|$ for 6 kinds of acceptors in the Ruthenium-modified azurin. The data are obtained by the MD simulations conducted by 10 ps. The dotted line is the exponential function with a slope 0.51 Å^{-1} .

each plane. The other is the conformation with constructive interference where the sign of the currents in each plane does not change so much but the amplitude of the currents varies considerably. The conformations with destructive interference appear when $|T_{DA}|$ is relatively small, while the conformations with constructive interference appear when $|T_{DA}|$ is large.

4. Photoisomerization Mechanism of Rhodopsin

Next, we go to the problem of *cis-trans* photoisomerization reaction of the retinal chromophore of rhodopsin. There have been a lot of investigations of the *cis-trans* photoisomerization mechanism so far [33–37]. However, detailed analysis of the molecular mechanism was not successful. Under such situation, recently two prominent achievements were made. One is the determination of three-dimensional structure of rhodopsin by X-ray crystallographic analysis by Palczewski *et al.* [38]. The other remarkable achievement came from the development of quantum chemistry [39]. By the full sa-CASSCF calculations, they searched the potential energy minimum root of the excited state of polyene Schiff-base as a function of twisting around the central double bond by allowing the relaxation of bond length. Then,

they found a conical intersection point at the twisting angle around 90° . Therefore, the photoisomerization is much easy through the conical intersection point.

Based on these achievements, we make MD simulation for the *cis-trans* photoisomerization of rhodopsin. Since the bond-stretching motion is much faster than the bond-twisting motion, we assume that the bond length can be well adjusted so that the conical intersection is realized when the twisting angle approaches 90° . Then, we adopt the barrierless excited state potential energy surface at around 180° which drops down to the ground state potential surface after about 60° for the twisting around the $C11=C12$, $C9=C10$ and $C13=C14$ bonds of the protonated Schiff base of retinal (Mt. Fuji potential).

The calculated results of the twisting angle of the chromophore as a function of time are shown in Figure 3. The $C11=C12$ bond rotates counterclockwise ultrafast and smoothly, from 10° (*cis* form) to about 140° in about 130 fs after photoexcitation (0 fs), essentially performing *cis-trans* photoisomerization. Moderate increase of the twisting angle about $C11=C12$ continues until about 160° at 450 fs and slow increase follows until about 165° at 2 ps. During this process, we observe that the other bonds close to the $C11=C12$ bond, especially $C9=C10$, $C10-C11$, $C12-C13$, $C13=C14$ bonds, are simultaneously twisted clockwise. We call this cooperative photoisomerization mechanism twist sharing mechanism. It is seen that the twisting around $C9=C10$ is much correlated with that around $C11=C12$. Its initial twisting angle (180°) decreases smoothly down to about 140° at 130 fs and it increases moderately up to about 150° until 450 fs and it increases slowly up to about 160° until 2 ps.

In this reaction, only the $C11=C12$ bond is isomerized and the intermediate of deformed all-*trans* form is obtained. If the $C9=C10$ bond which is partially twisted clockwise by about 40° until 130 fs continued to be twisted, we should obtain 9-*cis* chromophore as a result of photoisomerization. This was the story of the bicycle pedal model. However, it did not happen. The reality was that $C9=C10$ was prohibited to twist over 40° clockwise.

In order to resolve the mechanism why the rotation around the $C9=C10$ was blocked on the way, we did the excited state MD simulation, without changing the charge distribution on the chromophore from that of the ground state. The result was that the same *cis-trans* photoisomerization took place as above, indicating that the specificity of the *cis-trans* isomerization is not due to the change in the Coulomb interaction with the protein after photo-excitation but is due to the steric effect.

We searched which amino acids play a significant role in the *cis-trans* photoisomerization of the chromophore. We calculated the intrinsic movement of each atom due to only the photoexcitation, by subtracting the thermal motion which might occur in the ground state [40]. We selected six amino acids which showed large intrinsic movements. The retinal chromophore is neatly surrounded by these amino acids. Especially, Thr 118 is located near the C9-methyl of the chromophore. In order to identify the role of Thr 118, we made excited state MD simulation by ad-

Figure 3. The time course of the twisting angles of the retinal chromophore of rhodopsin. The data are obtained by the MD simulations and averaged for ten times run. At time 0, the chromophore is excited.

opting transparent Thr 118 which means that there is no van der Waals interaction potential and no partial charge in Thr 118 residue. In this case, we obtained 9-*cis* form of the chromophore by the excited state MD simulation, in accordance with bicycle pedal model. Indeed, we observe that Thr 118 blocks rotational motion of 10-H around the C9=C10 bond by the steric interaction with Thr 118. We also did excited state MD simulation using C13-desmethyl chromophore where 20-methyl is replaced with H and so the steric hindrance between 20-methyl and 10-H is much reduced. In this case, we obtain 13-*cis*, 9-*cis*, 11,13-*dicis*, 9,13-*dicis* as well as *all-trans* form of the chromophore. Therefore, we can say that rigid specificity of the *cis-trans* photoisomerization of rhodopsin is destroyed if the steric hindrance between 20-methyl and 10-H is released. Location of the two residues Ala 117 and Ala 292 around 20-methyl is important for conducting rotation motion of the 20-methyl in the counterclock way smoothly. In the model structure produced by Pogozheva [41], Ala 292 locates at the position that it pushes the 20-methyl to the Ala 117 side so strongly that the twisting direction of C12–C13 is opposite to that of X-ray structure. As a result, the excited state MD simulation using the Pogozheva model yielded 9-*cis* form of the chromophore, in accordance with bicycle pedal model. The role of Trp 265, Tyr 191 and Tyr 268 is mostly to fix the position of ionone ring and partly make a wall for the twisting around C9–C10.

Concluding the above results, the specific photoisomerization which is absolutely requisite for the photo-signal receptor is made possible by a very fine tuning of the configuration of the amino acids in the binding pocket of opsin.

Next, we address the origin of the high sensitivity of visual excitation. At the 90° twisting of the C11=C12 bond in the course of photoisomerization, twisting energy of the other bonds amounts to about 20 kcal mol⁻¹. 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 20 kcal mol⁻¹ and the thermal isomerization rate is decelerated by 10⁻¹⁴ times than that in solution. The *cis-trans* photoisomerization in rhodopsin takes place ultrafast in about 150 fs. Combining the above facts, we obtain extremely high specificity 10⁻²³ of photoreception of rhodopsin.

5. Conclusion

In this paper, we have shown how an intimate interplay between photobiology and physics is important, by examining three typical examples. In the energy transfer in the photosynthetic antenna complexes, we should not take it granted that an exciton mechanism will work simply because the excitonic coupling strength U is considerably large, or similarly we should not take it granted that Förster mechanism will work simply because U is rather small. From the quantum-statistical study of the energy transfer, we can say that what kind of energy transfer takes place depends on the magnitude of decay factor γ of the memory function as well as U . Examining the excitonic state of LH2 of *Rhodospseudomonas acidophila*,

we found that the energy transfer mechanism of the intra-ring of B850 is possibly the intermediate coupling mechanism with the exciton domain length (coherence length) of 4–5 BChl units. The energy transfer mechanism from B800 to B850 is also possibly the intermediate coupling mechanism. We have described some features of the energy transfer by the intermediate coupling mechanism, which might be helpful to observe experimentally.

How we should describe the electron tunneling root through protein media in the electron transfer of biological systems has been a problem of much debate for a long time. The pathway model in which much of chemical bonds is used is intuitively rational and easy to visual description. On the other hand, the significant role of the donor-acceptor length for determining the electron transfer rate is emphasized in the Dutton model. We have shown that the pathway model is based on the microscopic description of the electron tunneling root and that the Dutton model is based on the statistical averaged model of the tunneling current and then the factor of the donor-acceptor length becomes manifest. Both models do not contradict each other but they stand on the different level of description of the tunneling root. In such a way, we succeeded in consolidating the two models by taking statistical average of the inter-atomic tunneling currents. We have also investigated how the electron tunneling matrix element $|T_{DA}|$ is changed by the conformational fluctuation of protein. We have seen that there are two kinds of conformations: one having destructive interferences of inter-atomic tunneling currents leading to relatively small value of $|T_{DA}|$ and one having constructive interference leading to relatively large $|T_{DA}|$. It appears that the constructive or destructive interference is not a property due to the local conformation but due to property of the long-range conformation. Its analysis must be made in future.

As the third typical example, we investigated the mechanism of *cis-trans* photoisomerization of the retinal chromophore of rhodopsin by the MD simulation using the crystallographic structure of rhodopsin. We find that the high specificity (only C11=C12 bond being rotated counterclockwise) of the *cis-trans* photoisomerization of rhodopsin is made possible by the two kinds of steric hindrances: one is the intramolecular steric hindrance between 13-methyl and 10-H and the other is the intermolecular steric hindrance between 10-H and Thr 118 and between 9-methyl and Thr 118. We found that when we use the rhodopsin model structure obtained by Pogozeva *et al.*, we obtain a result that C9=C10 bond is rotated counterclockwise and C11=C12 is rotated clockwise in accordance with the bicycle pedal model. We found that the C12–C13 bond in rhodopsin of the Pogozeva model is twisted in an opposite way to that of rhodopsin in the crystallographic model due to the rather strong steric interaction between Ala 292 and retinal chromophore in the former model. This fact indicates that the active site of rhodopsin is designed much delicately (in much less than 1 Å accuracy) to produce desirable conformation of the photointermediate in the *cis-trans* photoisomerization of rhodopsin. It appears that such delicacy is necessary to produce very high photosensitivity 10^{-23} in visual excitation.

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