COGITATIONES

On the Diffusion Model of Localized Exciton in the Photosynthetic System

In the molecular assemblage of chlorophyll A, in the photosynthetic apparatus, there is only local orderness but no long range orderness of molecular orientation. The absorption and fluorescence spectra of chlorophyll A in the photosynthetic system are not different in main features from those of chlorophyll A in the solution system, except for the shift of the spectra towards wavelengths longer by about 20 mμ than the spectra of chlorophyll A solution. It is well known that the local concentration of chlorophyll A amounts to 0.06 ~ 0.2 mole/l in the grana of the photosynthetic apparatus on the average[1].

Our present purpose is confined to seeking the molecular mechanism and the extent of the migration of excitation in the system of chlorophyll A molecular assemblage which may be thought of as a simplified model for the grana.

Generally speaking, the presence of a non-localized exciton brings the change of the shape of the absorption and emission spectra of the crystalline system as a result of 'exciton splitting' which comes from the physical condition that the frequency of inter-molecular energy transfer is larger than the frequency of molecular vibration, 10^{13} sec^{-1}. The complete periodicity of the crystal system excludes the presence of a localized excited state. However, if there are structural imperfections present, like the grana in the photosynthetic system, a localized excited state occurs in the system. Owing to the weak intermolecular interaction, such a localized excited state on a constituent molecule may move into other neighbouring molecules. The frequency of inter-molecular transfer of a localized excited state will be smaller than that of molecular vibration, and larger than the emissive frequency. Therefore, the excited state localized on a molecule is swung by the molecular vibration to lose the vibrational excess energy of the excited state, but allowed to transfer the electronic excitation to other neighbours during the lifetime of emission. The motion of an excited state which shows a high degree of localization in the molecular assemblage may be pursued as a quasi-particle, which is called a localized exciton, and may be likened to the Brownian motion of a particle in aperiodic molecular assemblage.

The aperiodicity of the molecular apparatus of the biological system will be one of the characteristic features in molecular level. It may naturally be expected that this important character will be reflected in the biological function of the apparatus. In the case of the photosynthetic system, the aperiodicity of chlorophyll A molecular assemblage in the grana allows the presence and the migration of a localized exciton in the system without special variations in absorption and fluorescence spectra. Here we must notice that the function of the photosynthetic apparatus exists not only in transporting the excitation to spatially different place in the system, but also in transforming the excitation into chemical energy through coupling with enzyme reaction. Therefore, the role of the migration of localized exciton must be elucidated from the wide point of view. In contrast with the non-localized exciton, the stability of the localized exciton against the external perturbation from the chemically reactive substances may be thought to be important in the photoreactive system like grana, because the migration of a localized exciton is guaranteed by the aperiodic molecular assemblage of chlorophyll A.

The dipole-dipole interaction between the j-th and the k-th molecules,

$$V_{jk} = \frac{1}{\varkappa R_{jk}^3}\left\{\boldsymbol{\mu}_j \cdot \boldsymbol{\mu}_k - 3\frac{(\boldsymbol{\mu}_j \cdot \boldsymbol{R}_{jk})\,(\boldsymbol{\mu}_k \cdot \boldsymbol{R}_{jk})}{R_{jk}^2}\right\}, \qquad (1)$$

is taken as the inter-molecular interaction which brings the energy transfer, where $R_{jk} = |\boldsymbol{R}_j - \boldsymbol{R}_k| = |\boldsymbol{R}_{jk}|$ is the inter-molecular distance, $\varkappa$ is the dielectric constant, and $\boldsymbol{\mu}_j$ and $\boldsymbol{\mu}_k$ are the dipole moment operators of the j-th and k-th molecules. Then the transfer probability of excitation in the first electronically excited state from the j-th molecule to the k-th one per unit time is given in the form of

$$W_{jk} = \frac{1}{\tau_0}\left(\frac{R_0}{R_{jk}}\right)^6, \qquad (2)$$

where τ_0 is the optical lifetime of the first excited state, and R_0 is called the critical distance of the energy transfer, at that distance $R_{jk} = R_0$, the transfer frequency W_{jk} becomes equal to the emissive frequency $1/\tau_0$. In Eq. (2), R_0^6 for the couple of the energy donor and acceptor for the same kind is given in the various but equivalent forms, according to the several authors, on the assumption of random molecular orientation as the followings:

$$R_0^6 = \frac{3000 \cdot \ln 10}{32\,\pi^6\, n^4\, N}\int \frac{f(\tilde{\nu})\,\varepsilon(\tilde{\nu})}{\tilde{\nu}^4}\,d\nu, \qquad (2a)$$

(after FÖRSTER[2])

$$R_0^6 = \frac{3}{4\pi}\left(\frac{\hbar c}{n}\right)^4 Q\int \frac{f(E)\,A(E)}{E^4}\,dE, \qquad (2b)$$

(after DEXTER[3])

$$R_0^6 = \frac{3}{4\pi}\left(\frac{\bar{\lambda}}{2\pi n}\right)^4 \bar{\sigma}, \qquad (2c)$$

(after GALANIN[4])

or in the more convenient following form in which the mirror-image relation with respect to an axis of wave-number $\tilde{\nu} = \tilde{\nu}_0$ between the absorption and fluorescence spectra is used:

$$R_0^6 \simeq \frac{3 \cdot 10^6 \cdot (\ln 10)^2\, c\,\tau_0}{8\,\pi^4\, n^2\, N^2\, \tilde{\nu}_0^2}\int \varepsilon(\tilde{\nu})\,\varepsilon(2\tilde{\nu}_0 - \tilde{\nu})\,d\tilde{\nu}, \qquad (2d)$$

(after FÖRSTER[2])

where N is Avogadro's number, $\hbar$ is Planck's constant divided by 2π, c is the light velocity in vacuum, n is the refractive index of medium, E is the energy which is equal to $hc\tilde{\nu}$, being $\tilde{\nu}$ the wave-number in cm^{-1}, $f(\tilde{\nu}$ or $E)$ is the normalized function characterizing the shape of fluorescence spectrum, $\varepsilon(\tilde{\nu})$ is the decadic molar extinction coefficient at $\tilde{\nu}$, Q is the total absorption cross section which is related by $Q \cdot A(E) = \sigma(E)$ with the absorption cross section of energy E, in which $A(E)$ is normalized so that $\int A(E)\,dE = 1$ and characterizes the shape of the absorption band, $\bar{\lambda}$ is the mean wave-length in the overlap region between the absorption and fluorescence spectra, and $\bar{\sigma} = Q\int f(E)\,A(E)\,dE$ is the mean absorption cross section in cm^2 in the same overlap region as the above. It has been noted that Eq. (2) is valid for the case of the weak coupling between the energy donor and the acceptor[5], or, in other words, for the case of 'slow energy transfer' in FÖRSTER's sense[2].

The following numerical values for the (methanol or ether) solution system of chlorophyll A are obtained by

[1] E. RABINOWITCH, *Photosynthesis* (Interscience Publ., New York 1945), vol. 1, p. 412.
[2] TH. FÖRSTER, Radiation Research, Supplement 2, 326 (1960).
[3] D. L. DEXTER, J. Chem. Phys. *21*, 836 (1953).
[4] M. D. GALANIN, Zhur. Eksptl. i. Teoret. Fiz. *28*, 485 (1955).
[5] N. TAKEYAMA, Nature *191*, 1359 (1961).

means of graphical integration of the overlap area between the absorption and fluorescence bands:

$$\int_0^\infty \varepsilon(\tilde{\nu})\,\varepsilon(2\,\tilde{\nu}_0 - \tilde{\nu})\,d\tilde{\nu} = 2.15 \times 10^{12}\ \mathrm{cm}^3,$$

$$\tilde{\nu}_0 = 15 \times 10^3\ \mathrm{cm}^{-1}, \text{ and } \tau_0 = 1 \times 10^{-8}\ \mathrm{sec}.$$

By making use of Eq. (2d), R_0 is found to be 6.31×10^{-7} cm, where $n = 1.5$ was used. In the photosynthetic system, *in vivo*, the absorption cross section was experimentally estimated by ARNOLD and OPPENHEIMER[6] as

$$Q \text{ (for chlorophyll A } in\,vivo) = 0.5 \sim 1.4 \times 10^{-16}\ \mathrm{cm}^2.$$

Then, by making use of the values

$$\bar{\sigma} \simeq 1 \times 10^{-16}\ \mathrm{cm}^2,\ \bar{\lambda} \simeq 68 \times 10^{-6}\ \mathrm{cm}, \text{ and } n = 1.5,$$

and of Eq. (2c), R_0 for the couple of *in vivo* chlorophyll A-chlorophyll A can be estimated approximately as $6.3_3 \times 10^{-7}$ cm. Thus it is found that the values of R_0 for both couples *in vivo* and *in vitro* of chlorophyll A-chlorophyll A are nearly equal to about 63 Å. This value is used hereafter in the calculation of the diffusion constant and the diffusion length of a localized exciton in chlorophyll A molecular assemblage, and also in the calculation of the frequency of energy transfer.

Let us take the chlorophyll A molecular assemblage whose density of chlorophyll A per cm^3 is $\varrho_m = [C] \times N \times \times 10^{-3}$, where $[C]$ is the concentration of chlorophyll A in mole/l and N is Avogadro's number.

It may be said that the diffusion of localized excitons in a macroscopic level results from the Brownian motion of a quasi-particle of the excitation in aperiodic molecular assemblage.

When $P(\boldsymbol{R}, t)$ is the probability that an exciton is found in the position R in the system at time t, the diffusion motion of the localized exciton[7] is described by

$$\frac{\partial}{\partial t} P(\boldsymbol{R}, t) = D_e\, \nabla^2\, P(\boldsymbol{R}, t), \tag{3}$$

where the diffusion constant of the localized exciton is

$$D_e = \frac{1}{6} \cdot \frac{\langle R^2 \rangle}{\tau}, \tag{3a}$$

where $\langle R^2 \rangle$ is the mean square of the displacement from the site $\boldsymbol{R}$ to the site $\boldsymbol{R}'$ of the localized exciton, and $R = |\boldsymbol{R} - \boldsymbol{R}'|$, which is given by

$$\langle R^2 \rangle = \int_{R_u}^\infty R^2\, W(R; \tau)\, dv \tag{3b}$$

with $dv = 4\,\pi\,R^2\,dR$.

Here R_u is the effective lattice constant of the assemblage given by the relation of

$$\frac{4\,\pi}{3} R_u^3 = \varrho_m^{-1}. \tag{3c}$$

In Eq. (3b), $W(R; \tau)$ is the transfer probability of a localized exciton during time interval τ for the displacement R, and normalized so that

$$\int_{R_u}^\infty W(R; \tau)\, dv = 1. \tag{3d}$$

By making use of Eq. (2), this can be written as

$$W(R; \tau) = \frac{\tau}{\tau_0} \cdot \left(\frac{R_0}{R}\right)^6 \cdot \varrho_m. \tag{3e}$$

By Eqs. (3d) and (3e), we obtain the equation

$$\frac{1}{\tau_t} = \left(\frac{4\,\pi}{3}\right)^2 \cdot \frac{R_0^3}{\tau_0} \cdot N^2 \cdot 10^{-6} \cdot [C]^2, \tag{4}$$

which gives the frequency of inter-molecular energy transfer in the system of the concentration $[C]$. The critical concentration $[C_0]$ of energy transfer, at that concentration τ_0 is equal to τ_t (transfer time), is obtained from

$$\left(\frac{4\,\pi}{3}\right)^2 \cdot R \cdot N^2 \cdot 10^{-6} \cdot [C_0]^2 = 1 \tag{4a}$$

as $[C_0]$ (for chlorophyll A) $= 1.57 \times 10^{-3}$ mole/l. If the average lifetime of fluorescence $\tau_{fl} = \eta \cdot \tau_0$ is taken instead of τ_0, where η is the quantum yield of fluorescence and $\eta = 0.3$ for chlorophyll A solution system, we obtain the value of $[C_0] = 2.86 \times 10^{-3}$ mole/l.

According to WEBER's experimental data[8], $[C_0]$ is found to be about 1.7×10^{-3} mole/l.

By making use of Eqs. (3a), (3b), and (3e), the diffusion constant of a localized exciton becomes

$$D_e = \frac{1}{2}\left(\frac{4\,\pi}{3}\right)^{4/3} \cdot \frac{R_0^6}{\tau_0} \cdot \varrho_m^{4/3} = \frac{1}{2}\left(\frac{1}{\tau_t}\right) R_u^2, \tag{5}$$

which takes the usual physical interpretation of

$1/2 \cdot$ (transfer frequency) $\cdot$ (elementary jump distance)^2.

The diffusion length during the lifetime of a localized exciton τ_e is given by

$$l_e = \sqrt{D_e \cdot \tau_e} = R_u \sqrt{\tau_e / 2\tau_t}, \tag{6}$$

where τ_e is taken actually to be τ_0 or τ_{fl}.

The transfer frequency, the diffusion constant and the diffusion length of localized exciton in the chlorophyll A molecular assemblage are calculated with the parameter of chlorophyll A concentration (in mole/l) on the basis of the above-described equations, and summarized in Figures 1 and 2.

It is a remarkable thing that the region of chlorophyll A concentration in the grana fits in the allowed region of the transfer frequency of localized exciton which is limited by the inequalities of $10^{13}\ \mathrm{sec}^{-1} > 1/\tau_t \geqq 10^8\ \mathrm{sec}^{-1}$.

The calculated value of the transfer frequency of localized exciton for the system of chlorophyll A concentration, $[C] = 0.1$ mole/l, is

$$1/\tau_t = 4.07 \times 10^{11}\ \mathrm{sec}^{-1}.$$

When the optical lifetime, $\tau_0 = 1 \times 10^{-8}$ sec, of the localized exciton is taken, we obtain the root mean square of the number of energy transfers from

$$\bar{n}_t = \sqrt{\langle n_t^2 \rangle} = \sqrt{\tau_0/\tau_t} \tag{7}$$

as $\bar{n}_t = 64$ for the system of chlorophyll A concentration 0.1 mole/l. If the actual lifetime of fluorescence of chlorophyll A in the photosynthetic system[9] is taken as $\tau_{fl} = 1.5 \times 10^{-9}$ sec instead of τ_0, $\bar{n}_t$ becomes about 25. However, considering the difference in quantum yield between the photosynthetic system and the solution system, $\eta = 0.03$ for the former and $\eta = 0.3$ for the latter respectively, $\bar{n}_t$ recovers about 78. Hence it may be said that $60 \sim 80$ hoppings of a localized exciton are possible in the linear dimension on the average. The experimental value for the number of energy transfers in the photosynthetic system is $\bar{n}_t = 141$, which was obtained by TEALE[10] from the measurement of the fluorescence depolarization of *Chlorella* with the aid of the modified WEBER's formula[11]. By this experimental value, the frequency of energy transfer is found to be

$$1/\tau_t = \bar{n}_t/\tau_{fl} = \frac{141}{1.5} \times 10^9 \simeq 1 \times 10^{11}\ (\mathrm{sec}^{-1}),$$

[6] W. A. ARNOLD and J. R. OPPENHEIMER, J. gen. Physiol. *33*, 423 (1950).

[7] N. TAKEYAMA, Exper. *17*, 43 (1961).

[8] G. WEBER, *Comparative Biochemistry of Photoreactive Systems* (ed. by M. B. ALLEN, Academic Press, New York 1960), p. 395.

[9] S. S. BRODY and E. RABINOWITCH, Proc. 1st Natl. Biophys. Conf. (ed. by H. QUASTLER and H. J. MOROWITZ, Yale Univ. Press, New Haven 1959), p. 110.

[10] F. W. J. TEALE, Biochim. biophys. Acta *42*, 69 (1960).

[11] G. WEBER, Trans. Faraday Soc. *50*, 552 (1954).

where there is no need to take $\overline{n_t^2}$ for $\overline{n}_t$, because the essential character of WEBER's formula exists in the linear dependence of the inverse of fluorescence polarization on the concentration. The obtained value of transfer frequency agrees with the above-calculated value in the order.

The extent of the migration of a localized exciton during its actual lifetime in the photosynthetic system amounts to 200 ~ 400 Å which is calculated from the diffusion length of Eq. (6), and is smaller than the average diameter of a granum by about 10^{-1} times. This value of the extent of energy migration is comparable with the experimental value 10^2 Å which was obtained by THOMAS, BLAAUW, and DUYSENS[12] from the relationship between the size and the photochemical reactivity for Hill reaction of fragments of spinach grana.

It may be said that the diffusion model of the localized exciton will be a powerful approach to the problem of the migration of excitation in an aperiodic molecular assemblage like the grana in the photosynthetic apparatus.

Zusammenfassung. Das Problem der Energieübertragung im photosynthetischen Apparat wird mit Hilfe des Diffusionsmodells von lokalisiertem Exciton untersucht.

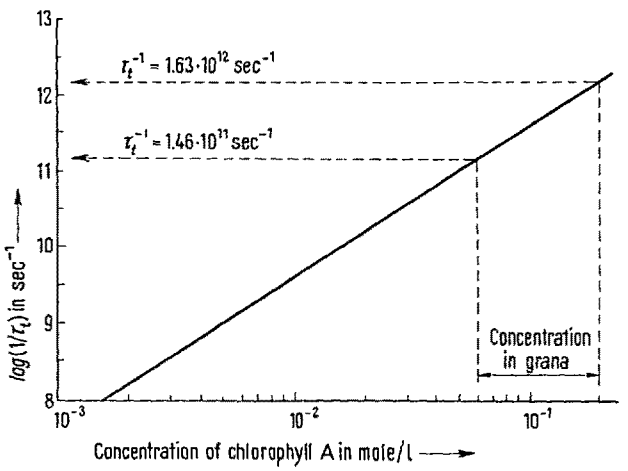

Fig. 1. The relationship between the logarithm of the transfer frequency ($1/\tau_t$) of localized exciton and the concentration of chlorophyll A, where the allowed region of the transfer frequency must be restricted by the inequalities 10^{13} sec$^{-1} > 1/\tau_t > 10^8$ sec^{-1}, according to the physical meaning of the localized exciton in an aperiodic molecular assemblage.

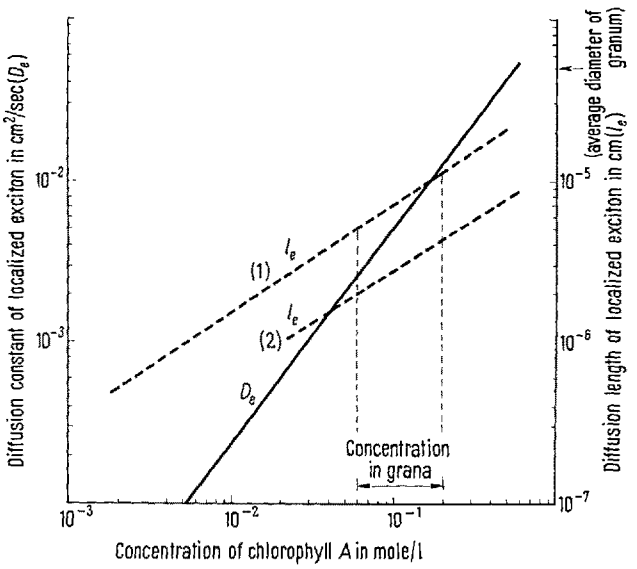

Fig. 2. The dependencies of the diffusion constant (D_e) and the diffusion length (l_e) of localized exciton in the chlorophyll A molecular assemblage on the concentration of chlorophyll A. (1): l_e during the optical lifetime $\tau_0 = 1 \cdot 10^{-8}$ sec. (2): l_e during the actual lifetime of fluorescence of chlorophyll A, $\tau_{fl} = 1.5 \cdot 10^{-9}$ sec, in the photosynthetic system.

Die Schwingungszahl der zwischenmolekularen Energieübertragung, der Diffusionskoeffizient und die Diffusionslänge des Excitons werden mit dem Parameter der Konzentration von Chlorophyll A gerechnet (Figur 1 und 2). Die gewonnenen Resultate ermöglichen den Vergleich mit experimentellen Daten.

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[12] J. B. THOMAS, O. H. BLAAUW, and L. N. M. DUYSENS, Biochim. biophys. Acta *10*, 230 (1953).

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PRO EXPERIMENTIS

The Recovery of Electron Microscope Grids

Several attempts have been undertaken in this laboratory to clean used copper electron microscope grids, the purchase of which considerably charges the budget. Boiling with detergents was ineffective, as well as the treatment with solvents because of the insolubility of electron bombarded sections and supporting membranes as well as of the carbon coatings.

The following procedure has been found effective and gives a reliable high yield of re-usable grids: the grids are placed on a piece of bronze mesh (about 2 × 4 cm mesh) and drawn through the upper third of a bunsen gas flame. The speed at which the bronze grid is drawn through the flame (about 10 cm/sec) is regulated so that the grids barely glow. After passage through the flame, the grids take on a blue-black colour. Immediately thereafter, the bronze mesh containing the grids is immersed in a nearby dish containing methyl- or ethyl alcohol. The oxide coating of the hot grids is immediately reduced, while the old membranes and the sections have been flamed off. It should be stressed that the flamed mesh should not be allowed to cool before immersion in the alcohol.

Zusammenfassung. Es wird ein einfaches Verfahren zur Reinigung elektronenmikroskopischer Trägernetzchen aus Kupfer beschrieben: Abflammen der Netzchen und nachfolgende Reduktion der gebildeten Oxydschicht durch Alkohol.

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