Photoresponsive Biodevices

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Abstract: Photoresponsive biodevices that work as photoswitches in biological reactions are reviewed. From a standpoint that the biodevices constitute biosystems, present status of techniques to assemble the biodevices into two-dimensional and three-dimensional systems are outlined. Finally, studies on electron transfers in proteins and polypeptides are briefly summarized as a possible method for communications between the biodevices.

1. Introduction

Light is a clean signal that can control chemical and biological reactions without disturbing total system significantly. Besides the photosynthetic system that utilizes photons as energy source, a variety of biological systems have been known to show photoresponsiveness. In addition, many chemists and biochemists have been attempting to modify photoinert biological molecules to acquire the photoresponsiveness. The purpose of the additional photoresponsiveness is very broad, i.e., for fundamental research on the mechanism of biological reactions, for biomedical applications, such as phototargeting in drug delivery, and for the development of biodevices. In these possible applications, the advantages of light signal are fully exploited. Light is advantageous over other signals (chemical, heat, electric, radiation, etc) in the following respects. (1) Photon may be transported through space without direct contact to the object. (2) Energy of a single photon may be selected by the wavelength. (3) Photon signal may be compressed as a short pulse of a few ten femtosecond. (4) Photon signal may be concentrated in a small spot of 1x1(μm^2) or less. Although applications of the photoresponsive biodevices are still very limited, their potentiality seems promising, because of the above characteristics. In this article, the author attempts to review works that have been made in the past two decades together with recent advances in this field.

The word "biodevices" may be interpreted in different ways. We believe that photoresponsive biodevices can be considered as parts of an intelligent biomolecular system in which external information in the form of photon is transferred to other form of signals, such as chemical and biochemical substances or electrons. It is important for the development of biodevices not only to prepare a variety of biodevices but also to assemble them into a highly organized system. Finally, communications among the biodevices are essential. Thus, the development of the intelligent biomolecular systems requires following three steps.

(1) Functionalization of biomolecules to prepare biodevices.
(2) Construction of organized assembly from the biodevices.
(3) Communications between the biodevices.

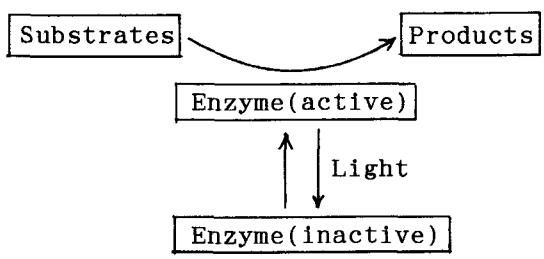
In the following, studies on the first subject are reviewed in the next section. Recent attempts towards the second and third issues are briefly reviewed in the third and forth sections.

2. Photofunctionalization of Biomolecules

Addition of photoresponsiveness to biomolecules was pioneered by Erlanger's group and Suzuki's group. The progresses until 1982 have been reviewed.[1]

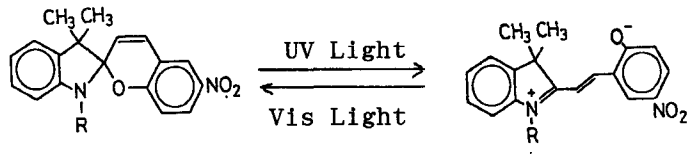
2-1. Direct Photomodulation of Enzyme Activity

The photomodulation of enzyme activity is an effective approach to switch biological reactions, because the change of activity of single enzyme molecule may induce changes in the a large number of product molecules. In other words, the photomodulation of enzyme activity by a single photon may be amplified in the form of a large number of product molecules.



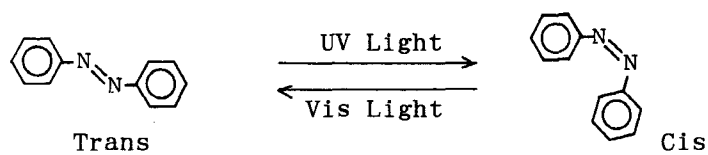
Direct modification of enzymes by a photochromic group (spiropyran derivative) was first reported by Namba et al[2]. The spiropyran group undergoes reversible photoisomerization between

a colorless spiropyran form and a colored photomerocyanine form as illustrated below.



Spiropyran form Photomerocyanine form

They attached spiropyran derivatives covalently to amylase and found that the enzyme activity decreased under photoirradiation. Karube et al[3] used membranes carrying spiropyran derivatives to immobilize enzymes and found that the enzyme activity can be controlled by photoinduced change in the hydrophilic character of the medium. Aizawa et al[4] attached spiropyran derivatives to a variety of enzymes and showed that a reversible photoregulation of enzyme activity can be achieved. The origin of the photoregulation is attributed to the alteration in hydrophilicity-hydrophobicity of the protein microenvironments. Very recently, Willner, et al[5] reported a photoregulation of papain activity through anchoring azobenzene groups to the enzyme backbone. The azobenzene chromophore is known to be stable in the trans form. Under uv irradiation the trans form isomerizes to the cis form. The cis form returns to the trans form thermally or under irradiation with visible (blue) light. The photocycle can be repeated for many times



Trans Cis

Although photoreversible changes of enzyme activity has been observed in these modified enzymes, none of them showed zero activity in the less active states. Since the zero activity is essential for the on/off-photoregulation of biological systems, challenges toward it is desired. For this purpose a more sophisticated molecular design to modify the neighbor of the catalytic sites of enzymes will be needed. However, incorporation of artificial functional groups in the form of nonnatural amino acids at the specific site of an enzyme is impossible by any conventional protein engineering techniques. An approach toward

this line has been started by a combination of genetic recombinant technique, in vitro protein synthesis technique, and synthesis of nonnatural amino acids carrying photofunctional groups.[6]

2-2. Modulation by Photochromic Effectors

A group of low molecular weight compounds are known to regulate enzyme activity. For example, inhibitors occupy active site of enzyme competitively with substrates and, as the consequence, suppress the catalytic activity. Erlanger et al.[7] synthesized artificial inhibitors that carry azobenzene groups. The principle of the photoregulation by the photochromic inhibitor is illustrated in Figure 1.

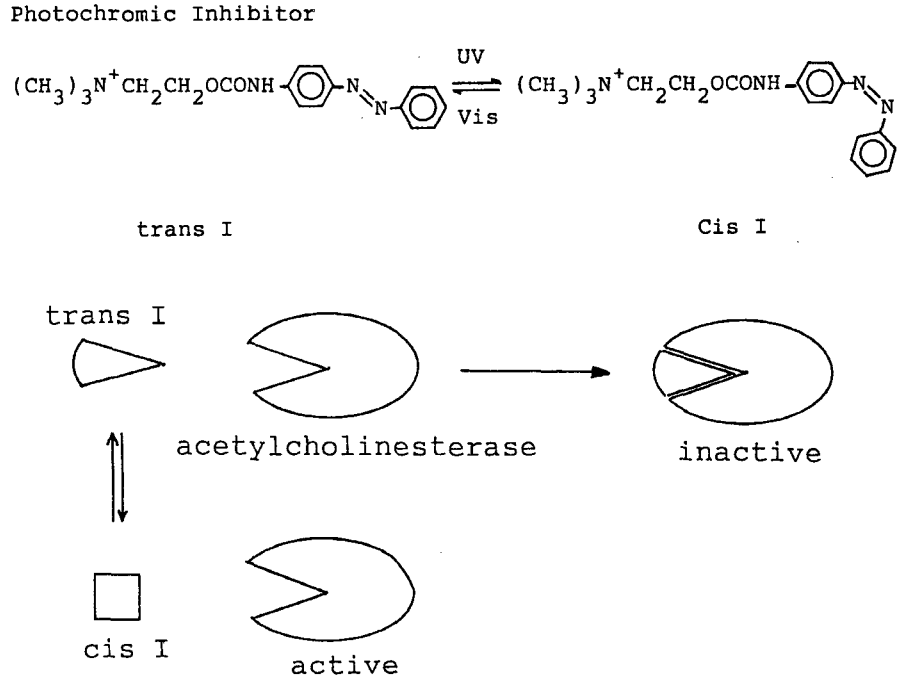


Figure 1. Photoregulation of enzyme activity by using photochromic inhibitor

They showed that the activities of serine esterases (chymotrypsin, trypsin, and acetylcholinesterase) can be photoregulated by the photochromic inhibitors. However, the change of enzyme activity was usually not very marked; for example, the activity of acetylcholinesterase changed from about 65% in the presence of trans inhibitor, to about 75% in the presence of cis inhibitor.

Erlanger and coworkers also synthesized a muscarinic antagonist carrying an azobenzene group (Bis-Q) and found that the agonist works as an open channel blocker of acetylcholine receptors (AChR) on the membrane of Frog heart or Electrophorus electricus.[8] The principle of the photoregulation of membrane potential is shown in Figure 2.

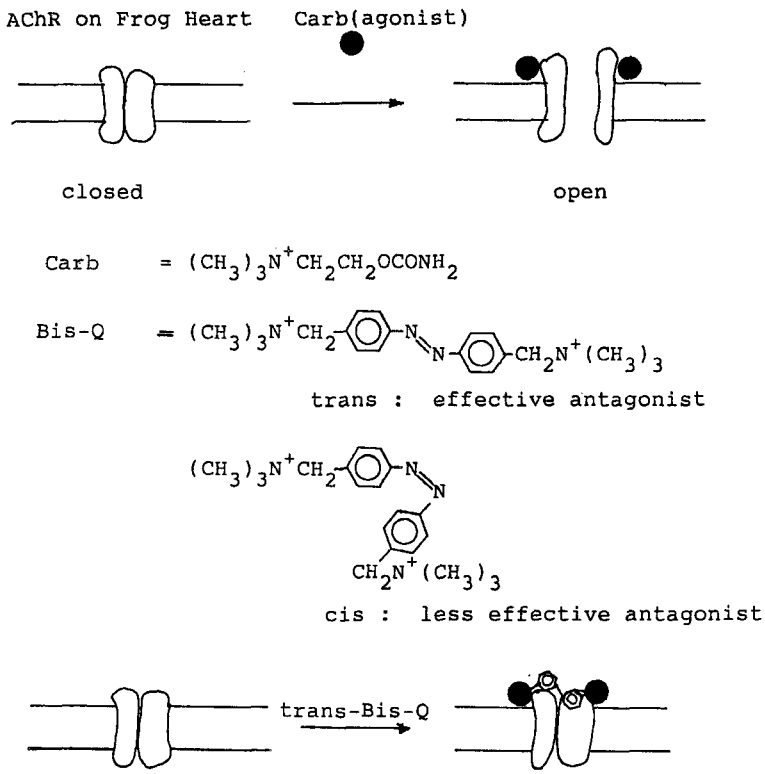
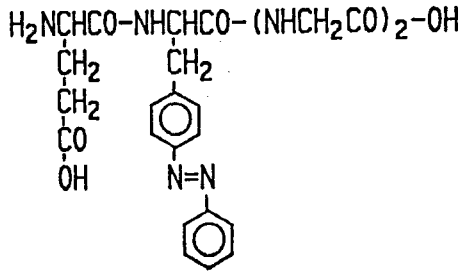


Figure 2. Photoregulation of membrane potential by using photochromic antagonist

Carbachol(Carb) is known to work as an agonist for AChR on membranes and induces electric current by K^+ ion flux. When Bis-Q was added to this system, the current decreased and the decrease was more marked for trans-Bis-Q then cis-Bis-Q. The result is tentatively interpreted in terms of the binding mode of trans-Bis-Q on the AChR as shown in Figure 2. Since the distance between two ammonium ions in trans-Bis-Q is appropriate for the closed form of AChR, the latter works as an effective antagonist. In this way, Erlanger and coworkers regulated the membrane potential by photoirradiation. However, for some unknown reason, the photoinduced change was not reversible.

2-3. Photocontrol of Antigen-Antibody Reactions

We have prepared monoclonal antibodies for an oligopeptide carrying an azobenzene unit.[9]



Mice was immunized with the peptide in the trans state and monoclonal antibodies were obtained. Fluorescence from one of the antibodies (Z1H01) was found to be quenched by the trans peptide very effectively, but not by the cis peptide (Figure 3). This indicates that the monoclonal antibody may bind only the trans peptide. As shown in Figure 4, the uptake and release of the peptide is reversible when the azobenzene group was irradiated alternately with 365-nm light (trans to cis) and with 435-nm light (cis to trans). The photocontrol of the antigen-antibody reaction was confirmed also from the HPLC analysis of the mixture. Similar photoreversible uptake and release were obtained when p-phenylazophenylalanine methyl ester (amino acid methyl ester carrying azobenzene group) was used as the hapten, instead of the tetrapeptide. Therefore, the antibody (Z1H01) is recognizing azobenzene group in the tetrapeptide. The principle of the photocontrolled antigen-antibody reaction is illustrated in Figure 5. From the measurement of transient absorption spectra of the hapten-antibody mixture, the azobenzene group was found to photoisomerize inside the binding site of the antibody. This indicates that the binding site is flexible enough to allow the isomerization.

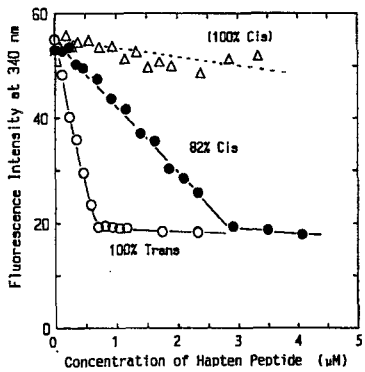
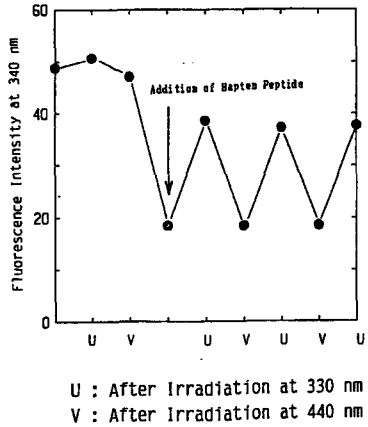


Figure 3. Fluorescence quenching of Z1H01 by the peptide carrying azobenzene group in trans form (○) and 82% cis form(●), and the calculated values for 100% cis form (△).



U : After Irradiation at 330 nm
V : After Irradiation at 440 nm

Figure 4. Changes of fluorescence intensity by irradiation with uv (U) and vis (V) light.

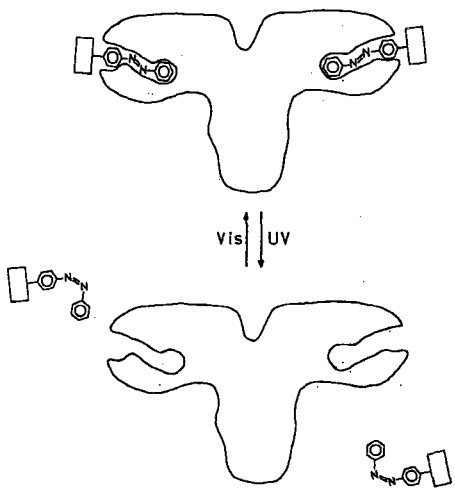


Figure 5. Principle of photoreversible uptake and release of hapten peptide carrying azobenzene group by the monoclonal antibody.

Monoclonal antibodies against molecules that are capable of (non-photochemical) trans-cis isomerization and those against quinone unit has been prepared in Schultz's group.[10,11] In the latter case, they showed that the uptake and release of the antigen-antibody reaction can be controlled by electric signals.

Harada, et. al prepared monoclonal antibodies for porphyrin unit.[12,13] The photocontrolled hapten-antibody reaction will be used as a photoswitch in a variety of biological reactions. For example, photocontrolled drug targeting or photoregulation of immune systems will be possible by the use of the photoswitch.

3. Organized Arrangement of Biodevices

3-1. Organized Assembly of Proteins

The photoresponsive biodevices described above have been studied mainly in solution. However, as stated in the first section, the biodevices will have to be assembled into biosystems on electrolodes, semiconductors, and other organic and inorganic sold surfaces. Not many attempts have been reported so far to prepare the organized assembly of biomolecules. Most of them exploit the self-assembling property of proteins.

Two-dimensional crystals of antibodies have been prepared on a monolayer of antigen molecules.[14] Similar strategy has been applied to the interaction between biotin lipids and Streptavidin in monolayers.[15] A general technique to prepare 2-D dimensional crystals of proteins has been searched for by using LB technique.[16] The development of common and facile method to obtain 2-D crystals may facilitate crystallographic analysis of proteins and thus accelerate preparation of artificially functionalized proteins by protein engineering techniques. The thin film of 2-D protein crystals themselves are novel bioelectronic devices.[16]

Hamachi, et al[17] found that a layered arrangement of Myoglobin molecules is attained by using synthetic bilayer membranes. Kunugi and coworkers[18] immobilized Bacteriorhodopsin inside polymer matrix under electric field. They observed generation of photocurrent only when the protein is oriented under high electric field in the matrix. The vectorial arrangements of proteins are said to be essential in exploiting photoelectronic property of proteins.

In these studies, however, only a single type of protein is assembled to form an organized system by utilizing self-assembling property of the protein. For an assembly of several types of proteins to construct functional biosystems, a novel technique will be required. The use of techniques developed for atomic-order microscopy (scanning-tunneling microscopy and atomic-force microscopy) will be one candidate and the techniques for microelectronics (photolithography and electron-beam lithography) are the other candidate.

3-2. Microphotopatterning of Peptides and Proteins

As described above, one of the advantages of light signal is its high spatial selectivity. Therefore if some photoresponsive biomolecules are placed on a 2-dimensional space and the surface is irradiated through a photomask, a pattern of the biomolecules will result. Fodor, et al.[19] performed solid-phase peptide synthesis using photolabile protecting groups on an aminosilylated glass surface. They succeeded to link 1024 different peptides on the surface by repeated cycles of photodeprotections under photomasks and additions of protected amino acids with coupling reagents. They showed that particular peptide molecules are attached in a particular spot on the glass surface by using antibody molecules that specifically bind a particular peptide. This is a novel technique to arrange a variety of peptides on a surface with an ordered pattern and to arrange a variety of antibody molecules through specific binding. However, a limitation of the technique is also evident. Since the resolution of photopatterning is on the order of submicrons, the above technique cannot be extended to molecular-scale assembly of peptides and proteins.

4. Electronic Communications in Polypeptides and Proteins

Despite the rapid progress in biochemical sciences the mechanisms of receiving, transducing, and storing information in biological systems are not yet understood in detail. However, it is almost certain that the biological information is processed by protein devices incorporated in biological organizates. Therefore, if one intends to develop artificial bioelectronic systems that mimic biological information processing, it is natural to start with a study on electron transfers in peptides and polypeptides and those between proteins. In this section, the author reviews basic studies on electron transfers in proteins, in protein-protein complexes, and in model polypeptides.

4-1. Electron Transfer Inside a Protein and Inside a Protein-Protein Complex[20,21]

Proteins that are participating biological electron transfers have been modified to attach electron donors or acceptors covalently. Intraprotein electron transfers are studied on the modified proteins to gain insights on the mechanism of biological electron transfer. For example, Cytochrome c that participates electron transport in mitochondria has lysine and histidine units on its outer surface. These units can be used as ligands for

metals, such as Ru^{2+} and Co^{2+}. The electron transfers between the heme group and the metal complexes have been studied. Some of rate constants measured on the modified proteins are collected in Table 1. It is evident that the electron transfer occurs across a distance of 10-20 Å within a few ten milliseconds. However, in some cases, the electron transfer is very fast. The principles that govern the electron transfer process in proteins are, therefore, is still unclear. Basic studies in more simple synthetic systems where the locations of the electron donor and acceptor, the distance and orientation of the chromophores, and the paths of the electron transfer are clearly defined, are needed.

In the respiratory and photosynthetic systems, the redox proteins usually reside in(on) the membranes as complexes with other proteins and electrons move along the sequence of proteins. Studies on electron transfers in protein-protein complexes is important in designing artificial biological devices. Table 2 lists rate constants of electron transfers between proteins that are associated to form tight complexes.

Table 1. Intramolecular Electron Transfer in Modified Proteins

Protein	Modification[a]	ET route	Distance(Å)	Driving Force(V)	Rate (s^{-1})	Ref.
Cyt c	^{33}His-RuL_5	Ru^{II}-Fe^{III}	12-16	-0.19	82	1
Zn-Mb	^{48}His-RuL_5	$^3ZnP^*$-Ru^{III}	11.8-16.6	-0.8	7×10^4	2
	^{81}His-RuL_5		18.8-19.3		85	
	^{116}His-RuL_5		19.8-20.4		89	
	^{12}His-RuL_5		21.5-22.3		100	
Pd-Mb						3
Zn-Cytc	^{33}His-RuL_5	$^3ZnP^*$-Ru^{III}	11.8	-0.82	7.7×10^5	4
Cyt c_{551}	^{47}His-RuL_5	Fe^{II}-Ru^{III}	7.9	-0.2	13	5
Cyt c	Asp or Glu-Co(diAMsar)	Co^{II}-Fe^{III}	15	-0.58	28	6

a) L=(NH_3)

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Table 2. Interprotein Electron Transfer in Protein Complexes

Protein Complex	ET route	Distance(Å)	Driving Force(V)	Rate (s^{-1})	Ref.
Cyt c/ccp[a]	Fe^{II}(Cyt c)-Fe^{IV}(ccp)	22		150	1
Zn,Fe-hybrid Hb	^3Zn - Fe^{III}	25	-1.25	60	2
Zn-Cyt c/ccp	^3Zn(Cyt c)-Fe^{III}(ccp)	17-18	-1.25		3
Cyt c/Cyt b_5	Fe^{II}(Cyt b_5)-Fe^{III}(Cyt c)	8.5	-0.2	1.6×10^3	4
	$^3ZnP^*$(Cyt c)-Fe^{III}(Cyt b_5)	8.5	-0.3	5×10^5	
	$^3Por^*$(Cyt c)-Fe^{III}(Cyt b_5)	8.5	-0.8	5×10^4	
	Por(Cyt c) -Fe^{III}(Cyt b_5)	8.5	-1.1	8×10^3	

a) ccp = Cyt c Peroxidase

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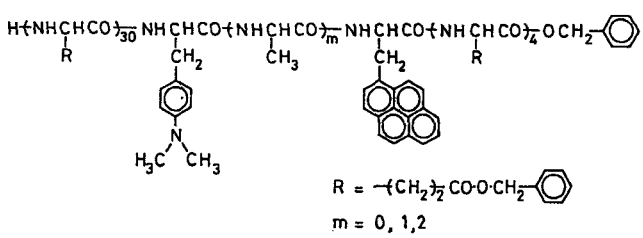
4-2. Electron Transfers on Model Helical Polypeptides[22,23]

From the studies on electron transfers in proteins and protein complexes, the electron was found to transfer across a distance of the order of 10-20 Å. However, the mechanism or the path of the electron transfer, i.e., through bonds or through space is sill unclear. For a basic study on electron transfer in polypeptide systems, helical polypeptides (I-m) carrying L-p-dimethylaminophenylalanine (electron donor) and L-pyrenylalanine (electron acceptor) were synthesized. The dimethylanilino (D) group and pyrenyl (P) group are separated by different number of alanine groups (m=0,1, and 2). The chemical structure and the predicted conformation of the three polypeptides are shown in Figure 6.



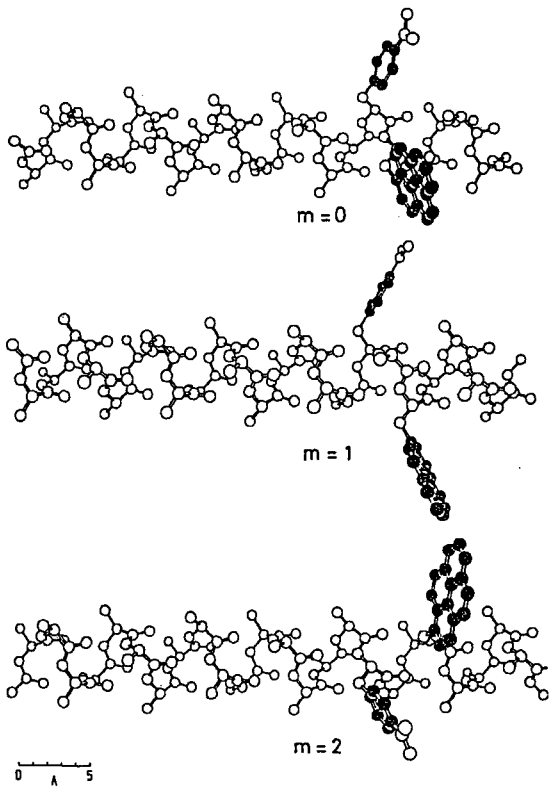


Figure 6. Conformations predicted for polypeptides carrying pyrenyl (P) and dimethylanilino (D) group.

It is well known that the D-P pair undergo photoinduced electron transfer after photoexcitation of P group. The rate of the photoinduced electron transfer were measured for the three polypeptides from the fluorescence decay profiles of the P group. The rate constants and the edge-to-edge distances between D and P group are: $k=1.9\times10^{7}$ (5.4 Å, m=0), $k=6.6\times10^{5}$ (9.4 Å, m=1), and 2.1×10^{7} (5.5 Å, m=2) at -20°C in trimethyl phosphate. Two major conclusions may be drawn from the above data. (1) Although the D-P pair of the m=2 polypeptide is separated by a larger number of bonds than that of the m=1 polypeptide, the electron transfer

rate is faster in the former polypeptide. This indicates that the electron transfer does not proceed via "though bond" mechanism, but via "though space" mechanism. (2) Although the relative orientations of D and P group in the m=0 and m=2 polypeptide are very different, the rate constants for the two polypeptides are about the same. This suggests that the rate of electron transfer is not much sensitive to the orientation of the relevant chromophores.

Taking advantage of rigid helical structure of oligoproline chains, the distance dependence of electron transfer rates have been studied on D-$(Pro)_n$-A systems with different D and A chromophores.[24-27] However, the rigidity of the oligoproline chain has been questioned.[25]

5. Concluding Remarks

Development of photoresponsive biodevices and their arrangement or organization are still at an infant stage and the utilization of these biodevices in microsensors and other bioelectronic systems will require further advances, especially, in the method for transporting electrons and other biosignals between the biodevices. However, our goal is very clear as seen in natural biosystems. An example is shown in Figure 7[28]

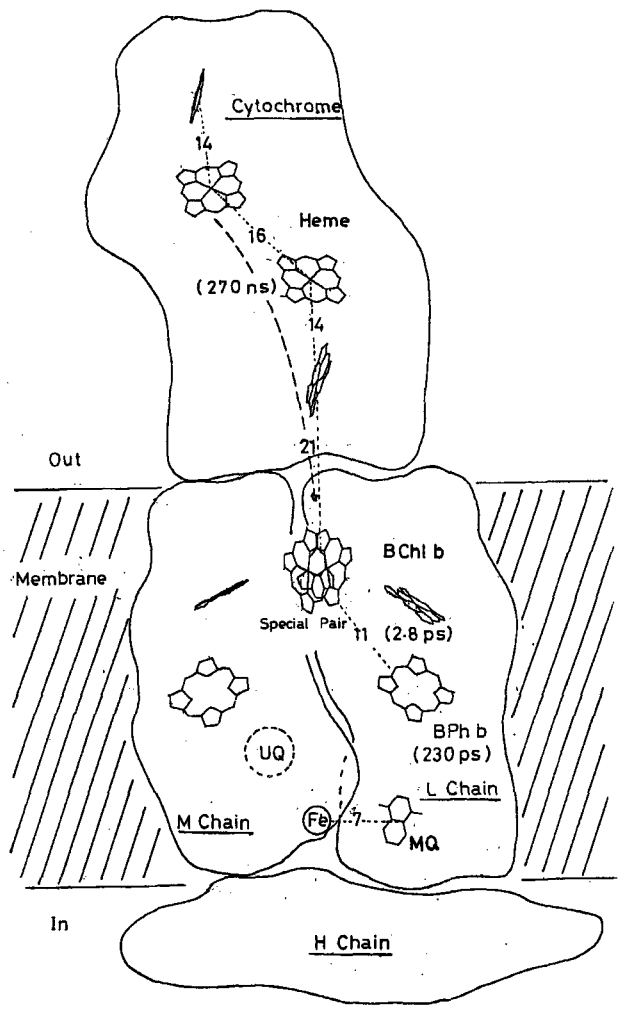


Figure 7. Chromophore arrangement in the photoreaction center of Rhodopseudomonas viridis (ref.28).

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