Current Topics in Biophysics, 2011, 34, 53-65

REVIEW
FLAVINS AS PHOTORECEPTORS OF BLUE LIGHT AND THEIR SPECTROSCOPIC PROPERTIES

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Received December 12, 2011; accepted December 23, 2011; published online December 30, 2011

This review describes 1) the development of studies on flavin photoreceptors as blue light photoreceptors in many living organisms: their kinds and functions; 2) the studies on spectroscopic properties of flavins, both their dimers and monomers; 3) nonradiative excitation energy transport in the presence of monomers and fluorescent/nonflurescent FMN dimers (excitation traps). The existence equilibrated luminescent FMN centers, energy migration and excitation sink to FMN dimers are taken into account.

INTRODUCTION

Flavins (FMN – flavin mononucleotide, FAD – Flavinadenin dinucleotide, R – riboflavin) (Fig.1) play extremely important biological role in oxireduction reactions taking place in respiratory chain, Krebs cycle, in pirogronian dehydrogenase system and other reactions (Stryer, 1995).

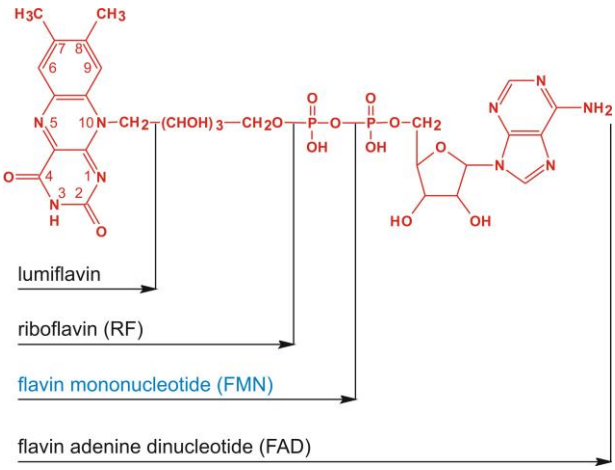

Fig.1.Chemical structure of flavins (based on Fg.1. by Grajek, 2007).

In natural biological structures flavins bind to peptide forming complexes known as flavinproteins, the most important part of which are flavin enzymes. Flavins as a part of enzymes take part in proton and electron transfer, leading among others finally to energy conversion in the organism. Reactions connected with the presence of falvins occur also in mitochondrial membranes, in cytoplasm and other parts of living organisms cells.

The second important function of flavins is their action as photoreceptors of blue light. Flavins absorb light in ultraviolet and visible spectral range. In the excited state they undergo easily photooxidation reactions which make them excellent candidates for playing a role of photoreceptors in different processes connected with the reaction of plants towards light. For example, through flavin enzymes light can regulate photosynthetic carbon and nitrogen metabolism. Excited flavins take part in the reactions of photorespiratory and nitrate reduction pathways. Flavins as photoreceptors take part also in the reactions of chloroplast translocations upon the effect of irradiation by light and in phototropic reactions. They take also part in the process of plants respiration. The action spectrum for the enhancement of respiration by light of the yellow *Chlorella* mutant (Kowallik, 1967; Kowallik & Kowallik, 1969) exhibits its maximum at 460 nm and 375 nm, similarly to the phototropism action spectrum and is also close to the absorption spectrum of flavins. Flavins excited to the triplet state take part in reaction catalized by flavin enzymes.

The photoreception phenomenon is widely studied in two directions. The first one concerns identification of the dye responsible for photoreception. The second one concerns investigation in which way information transferred by light is transformed in the cell and leads to a given morphogenetic process (morphogenesis – all reactions leading to the development of plants:

germination, growth, flowering etc.) or the movement reaction. These mechanisms are still not fully understood.

The aim of the present review is to show the development of studies on flavin photoreceptors, their current state, and in particular to draw the attention of scientific community to the investigations of spectral properties of monomers and dimers of flavins as well as excitation energy transport with the participation of flavin dimers. Such information can be very helpful in the explanation of the mechanism of photoreception processes in which flavins take part.

CHARACTERISTICS OF FLAVIN PHOTORECEPTORS

The potential role of different dyes as blue light protoreceptors was a subject of controversy in seventies and eighties. This subject concerned the role of carotenoids, flavins and pterine (Presti, Hsu & Delbrück, 1977; Ninnemann, 1980; Quiñones & Aparicio, 1990). As early as in 1975 Zurzycki (1975) has shown that the action spectrum of chloroplasts motion occurring upon the effect of light in the cell of *Funaria* is similar to that of flavin absorption (Zurzycki, 1975). This unknown blue light photoreceptor has been named then cryptochrome (Cry). To identify which dye is hidden under cryptochrome name the action spectra of numerous biological processes induced by blue light have been measured: DNA photoreactivation, chloroplasts motion, germination of fern spores, phototropism of plants, circadian rhythm in plants etc. The authors have shown that the action spectra of many organisms reflect more absorption spectra of flavins than those of carotenoids (Ninnemann, 1980; Haupt & Wagner, 1984; Quiñones *et al*.,1990). Gabryś has proved in many papers based on action spectra of chloroplast rotation in the alga *Mougeotia* that the role of cryptochrome is played by a flavin (Gabryś, Walczak &Haupt, 1985; Gabryś, 1986; Walczak, Gabryś, & Haupt, 1984).

Many other authors indicated also flavins as photoreceptors of blue light (Ninnemann, 1980, Lenci, Colombetti & Häder, 1983; Schmidt, 1984; Haupt *et al*. 1984; Löser & Schäfer,1986, Quiñones, *et al.*, 1990). Figure 2 shows the action spectra of several organisms presented originally in the work of Ninnemann (1980). Visible maxima correspond to the location of flavin absorption band maximum. Moreover, the action spectra possess a characteristic shoulder at the excitation wavelength close to 480 nm.

Rapid progress in the studies of photoreceptors started in 1993 with the initiation of genetic studies, which proved that the first cryptochrome Cry1 is the flavin (FAD). Next, the second flavin photoreceptor Cry2 has been discovered, which differed from the first one by the group binding the chromophores. At present several groups of flavin photoreceptors have been identified: cryptochromes, phototropins, BLUF photoreceptors, ZTL, FKF1 and LKP2 family of photoreceptors.

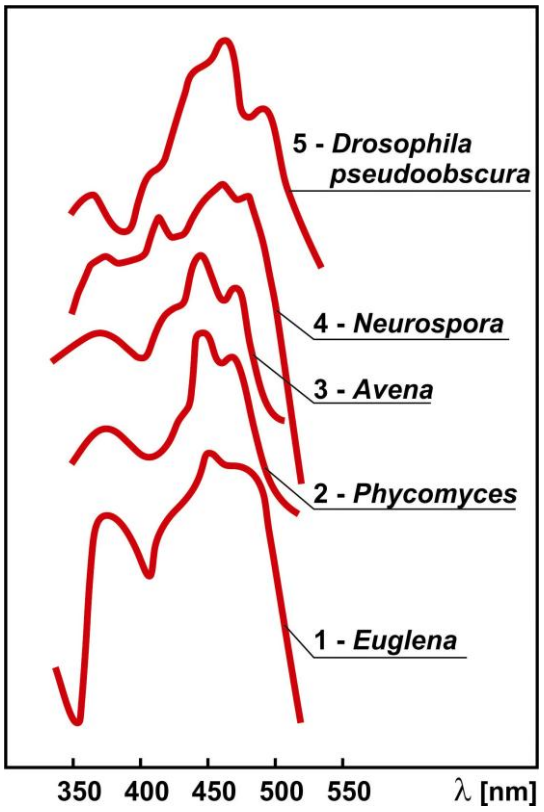

Fig.2. Selected action spectra for various blue light responses from Ninnemann (1980): phototaxis of *Euglena* (1), phototropism of *Phycomyces* (2) and Avena (3), photoinhibition of expression of circadian conidation rhytm of *Neurospora* (4), photoinduced delay shift of the circadian eclosion rhythm of *Drosophila pseudoobscura* (5), (based on Fig.1 by Ninnemann, 1980).

Cryptochromes

Cryptochromes are flavin photoreceptors, which regulate the grow and circadian rhythms generated by light in living organisms from bacteria and plants through human being (Partch, Clarkson, Ozgur, Lee & Sancar, 2005). They act as an integral part of central circadian oscillator in animal brains (so called molecular clock). They control also photomorphogenesis as receptors in plants reply to to blue light or UV radiation (Lin & Todo 2005). The peptide part of cryptochromes exhibits strong similarity of aminoacid sequence to photolyase - enzymes stimulated by light, which repair DNA. Plant and animal cryptochromes possess from 30 to 250 aminoacidic residues from C–terminal domains of the peptide chain (Partch *et al.*, 2005). Their three-dimensional structure is characterized by the presence of

a domain containing both α and β fragments. In the helical domain the access cage for FAD is a catalytic site for photolyase. It is thought that this part of a peptide is extremely important in the mechanism of cryptochromes action. (Lin *et al.*, 2005). Studies on functions and cryptochromes actions are very extensive (Lin, Yang., Guo, Mockler, Chen & Cashmore, 1998; Devlin & Kay, 1999; Guo, Duong, Ma & Lin, 1999; Lin, 2000). The same concerns molecular and biochemical differences between Cry1 and Cry2 (Zhu & Green, 2001) and conformational changes of cryptochromes upon irradiation by light (Partch *et al.*, 2005). The studies have concerned the photocycle of the cryptochrome photoreceptors (Liu B., Liu H., Zhong & Lin., 2010).

Phototropins

Later discovered flavin photoreceptors have been phototropins. This is a plant photoreceptor family of blue light and UV-A radiation (Briggs & Olnej, 2001) containing among others two types of photoreceptors Phot1 and Phot2 involved in phototropism, blue light-induced chloroplast relocation, stomatal opening and the inhibition of hypocotyl elongation. Phototropin possesses two domains at the N-termini called LOV1 and LOV2 each of them being a binding site for the FMN flavin. Their C termini contain, in turn, a serine/threonine protein kinase domain.

These are peptides regulated by the signals: light – oxygen- voltage. This forms the origin of LOV name (Light, Oxygen, Voltage). The properties of LOV1 and LOV2 domains have been thoroughly studied by Salomon and coworkers (Salomon, Chistie, Knieb, Lempert & Briggs 2000). LOV domains have the main absorption maximum at 360 nm and 450 nm as well as they exhibit their vibronic structure at 425nm and 475 nm. These spectra are similar to phototropism action spectra in higher plants. LOV1 and LOV2 domains contain five β-sheets and two α-helices as basic elements of their secondary structure (Salomon *et al.*, 2000)**.** From roentgenographic studies Crosson and Moffat (2001) have defined the LOV2 domain structure of phototropin module of fern photoreceptor *Adiantum* phy3, containing FMN in the peptide cage. They proved that due to the irradiation by blue light FMN covalently binds to cysteine. Sakai *et al*. (Sakai, Kagawa, Kasahara, Swartz, Christie, Briggs, Wada & Okada, 2001) proved that FMN binds in *Arabidopsis* in a noncovalent way within two LOV domains. They studied carefully the functioning of those photoreceptors not only in phototropism but also in chloroplasts movement upon light. The action of Phot2 photoreceptor as a controller of chloroplasts movement have been also shown by Jarillo et al (Jarillo, Gabryś, Capel, Alonso, Ecker & Cashmore, 2001). Widely discussed are the investigations of a photocycle of Flavin-binding domain LOV with the FMN transformations upon light. These changes concern photoinduced FMN-cysteine adduct formation (Swartz, Corchnoy, Christie, Lewis, Szundi, Briggs & Bogomolni, 2001; Fedorov, Schlichting, Hartmann, Domratcheva, Fuhrmann & Hegemann, 2003; Durr, Salomon & Rüdiger, 2005; Kottke Heberle, Hehn, Dick & Hegemann, 2003; Schüttrigkeit Kompa, Salomon, Rüdiger& Michel-Beyerle,2003; Tyagi, Penzkofer, Mathes & Hegemann, 2010). The authors show that the triplet state of FMN is responsible for the formation of flavin-cysteine adduct.

Kottke *et al.* (2003) describes the mechanism of reactions of LOV1 (*Chlamydomonas reinhardtii*) changes in the following way. The photoexcitation of the dark form of LOV1-447 causes the FMN transition to the excited singlet state, from which the intersystem crossing transition takes place to the triplet state of FMN (LOV1-715). This state assumes two forms: LOV1-715a and LOV1-715b. The first form transforms into LOV1-390 form within 800 ns, whereas the second one either transforms into that form directly within 4 microseconds or through LOV1-715a form. The formation of LOV1-390 is accompanied by the adduct formation (C(4a)-S and N(5)-H) FMN-cysteine, which degradates to the the dark of LOV1-445 during the reverse reaction. The studies of kinetics of photoproducts formation of LOV domains phot1 and phot2 have also been performed (Kasahara, Swartz, Olney, Onodera, Mochizuki, Fukuzawa, Asamizu, Tabata, Kanegae, Takano, Christie, Nagatani & Briggs, 2002).

BLUF ptotoreceptors

BLUF is a class of blue light photoreceptors. These are AppA flavoproteins possessing domains which bind FAD. The corresponding coding sequences have been found in genomes of photosynthetic bacteria, cyanobacteria and *Euglena* (Fukushima, Okajima, Shibata, Ikeuchi & Ithon, 2005). AppA flawoprotein acts under the blue light effect as an antirepressor of a photosynthesis gene expression in the purple bacterium *Rhodobacter sphaeroides* (Kraft, Masuda, Kikuchi, Dragnea, Tollin, Zaleski & Bauer, 2003). The studies of *Rhodobacter sphaeroides* flavoproteins have shown that AppA contains two domains, a Cys-rich carboxyl-terminal domain responsible for the isomerization of disulfide bond in PpsR and an amino-terminal domain which noncovalently binds the blue-light-absorbing chromophore FAD. These studies have shown the photoreactivity of that domain (Masuda & Bauer, 2002; Kraft *et al.*, 2003). The investigations of AppA domain photocycle have also been widely made (Fukushima *et al.*, 2005; Kraft *et al* 2003; Zirak, Penzkofer, Hegemann & Mathes, 2007). Gauden *et al.* (Gauden, Yremenko, Laan, van Stokkum, Ihalainem, van Grondelle, Hellingwerf & Kennis; 2005) have shown that both singlet and triplet states of excited FAD participate in

this photocycle. The studies of AppA domains with Roentgen radiation (Anderson Dragnea, Masuda, Ybe, Moffat & Bauer, 2005) indicate that the photoactivation can induce the reorganization in binding flavins with the peptide.

The ZTL, FKF1, LKP2 family of photoreceptors

The ZEITLUPE (ZTL) photoreceptors, Flavin-binding Kelch F-box1 (FKF1) and LOV Kelch Protein 2 (LKP2) proteins is a novel family of blue-light photoreceptors involved in modulation of the circadian clock and regulation of flowering (Demarsy & Fankhauser, 2009; Banerjee & Batschauer, 2005). These photoreceptors function in *Arabidopsis* and possess the N-terminal LOV domain followed by F-box moiety, and the C-terminal six Kelch repeats. The LOV domain of these peptides binds FMN chromophore and possesses similar photochemical properties like phototropion LOV domains. The Kelch forms four to six tandem repeats yielding a β-propler and may serve as the protein-protein interaction domain that recruits specific proteins for degradation. The degradfation response could also be light regulated (Banerjee *et al.*, 2005). FKF1 leads to the expression of CO, a central element of day-lenth-regulated flowering (Turck, Fornara & Coupland, 2008). CO transcription is regulated by day length while the protein is unstable in the dark and stabilized during the day (Turck *et al.*, 2008; Demarsy *et al.*, 2009). FKF1 regulates the stability of Cycling Dof Factor 1 (CDF1), which directly represses CO expresion. The LOV domain of FKF1 interacts with a plant-specific protein called GIGANTEA (GI), which is another positive regulator of CO expression. This interaction occurs upon blue light irradiation and it depends on the photoexcitation of LOV domain. ZTL interacts also specifically with GI during photoexcitation. GI expresion is clock controlled, thus this light-regulated interaction explains diurnal regulated accumulation of ZTL protein despite constitutive RNA expression.

At present the studies on flavin photoreceptors develop very dynamically. Rapid progress can be observed in the identification and characterization of photoreceptors, their structure and unusual photochemistry as well as their functioning (Briggs, Christie & Salomon 2001; Briggs *et al.*, 2001, Demarsy *et al.*, 2009). Photocycles are proposed during which FMN and FAD undergo transformations. However, the mechanism of signal transduction in photoreception phenomena in which flavins participate is far from being fully known.

SPECTRAL AND STRUCTURAL PROPERTIES OF FLAVIN PHOTORECEPTORS

Flavins and their photochemistry have been the subject of intensive studies for about eighty year (Kautsky& Bruijn 1931; Kuhn & Weygand 1934; Weber 1948; Flavins and Flavoproteins: 1966, 1968, 1971, 1976; Song 1971; Song, Moore & Kurtin 1972; Sun, Moore & Song et, 1972; Siódmiak & Drabent, 1973; Penzer & Radda, 1976; Drabent, 1977, 1979; Drabent, Mieloszyk & Siódmiak, 1984; Müller, 1981; Heelis, 1982). Since the sixties conferences devoted exclusively to flavins have been organized on a regular basis, i.e. „Flavins and Flavoproteins – International Symposium". In 2011 the 17th International Symposium on Flavins and Flavoproteins took place in at The University of California, Berkeley, USA.

Flavins have been very thoroughly investigated with the spectroscopic techniques. Their absorption, fluorescence, fluorescence excitation, phosphorescence and polarization spectra have been many times measured and their photophysical properties like emission anisotropy, solvatochromism and temperature effect on absorption and luminescence spectra, vibronic structure and intersystem crossing have been elaborated (Beinert, 1960; Kozioł, 1966, 1969; Song, 1971; Scola-Nagelschneider& Hemmerich 1972, Siódmiak & Frąckowiak, 1972; Song *et al*., 1972; Sun et al. 1972, Müller *et al.*, 1973; Visser, Ommen, Ark, Müller & Voors;, 1974; Drabent & Białłowicz, 1976; Drabent, 1977; Heelis, Parsons B, Phillips & McKellar, 1978; Heelis 1982; Bystra & Drabent 1982; Bystra, Drabent & Szubiakowska, 1983; Drabent *et al*.,1984; Drabent & Laczko, 1984; Grajek, Drabent, Żurkowska .& Bojarski 1984; 1986; 1990; Grajek, Żurkowska, Bojarski P., Kukliński, Smyk, Drabent & Bojarski C 1998; Grajek, 2003; Grajek, 2007).

Absorption spectra of flavins: FMN, FAD and RF are similar. FMN possesses characteristic absorption maxima at 445 nm, 370 nm, 265 nm and 220 nm as well as the fluorescence band with the maximum around 520 nm in water solutions and phosphorescence band at around 605 nm at low temperatures (below 150 K) in ethanol. The π-π character of first two transitions in absorption has been confirmed by theoretical calculations performed using quantum chemical methods (Song, 1969a; Song, 1969b; Grabe 1974, Sun *et al.*, 1972) as well as by experimental data obtained from polarization spectra in the excitation and emission band (Song 1969a; Siódmiak *et al.*, 1972; Song *et al*., 1972; Sun *et al*., 1972). Based on the theoretical calculations the existence of two π-π transitions at 280 nm and 260 nm (Sun *et al*., 1972) has been found. It has been also indicated that they can contribute to the observed maximum at 265 nm. Also the n-π transitions are possible in view of four free electron pairs at

nitrogen and oxygen atoms in the isoalloxazine ring (Sun *et al*., 1972; Sun & Song, 1973; Drabent *et al.*,1976). The spectral location of these transitions is, however, not fully known. Some authors suggest their appearance at 300 nm, 370 nm or 480 nm (Song 1971; Harders, Förster, Voelter& Bacher, 1974, Drabent,1979). The location of transition moments in absorption and the angle between them has also been investigated and determined among others by Johanssson *et al.* (Johansson, Davidson, Lindblom & Naqvi; 1979) and Eaton *et al.* (Eaton, Hofrichter, Makinen, Andersen & Ludwig, 1975). They obtained $\angle(\overrightarrow{M}_I, \overrightarrow{M}_{II}) = 39°$. Many spectroscopic and photochemical properties of flavins have been reported in a review by Heelis (1982), Drabent (1977) and Grajek (2007). The interactions of flavins with aminoacids and aromatic hydrocarbons have been studied in a number of works (Tollin 1968; Flavins and Flavoprotetns 1971; Müller, Mayhew & Massey, 1973; Song *et al*.1972; Ulbrych, 1979; Müller, 1981; Bystra 1984; Bystra, Drabent, Szubiakowska & Smyk, 1985; Mieloszyk, Drabent & Mieloszyk, 1989; Berg, Hoek & Visser, 2004). Flavins interact „stackingly" with the rings of aromatic compounds and aminoacids (Muñoz, Carmona, Hidalgo, Guardado & Balón, 1995).

Worthy mentioning are the structural studies and conformation dynamics studies of enzymes and flavin coenzymes (Narayanasami, Horowitz & Master, 1995; Estabrook, Shet, Fisher, Jenkins & Waterman, 1996; Miura, Maeda Arai, 2001; Guo *et al*., 2002; Berg & Visser, 2001)**.** Also the deactivation processes of flavins excited states are studied in view of their use in explanation of reactions dynamics in various biological systems. The formation efficiencies of radical pairs in the photoinduced charge transfer from tryptophan residues HEWL (hen-egg-white lysozyme) to FMN (Miura *et al*., 2003) have been determined. This latter process is accompanied by the quenching of the FMN singlet state. Two types of triplet state quenching in the FMN-HEWL system have been proposed: quenching through the diffusion of HEWL and FMN and quenching connected with the interaction of HEWL with FMN. Worthy of note are also the studies devoted to photochemical reactions occurring between the FMN in the excited triplet state and aromatic aminoacids (tyrosine, tryptophan and histidine connected with the electron and proton transfer. These investigations have been performed with the time-resolved nuclear magnetic resonance (Tsentalovich, Lopez, Hore & Tagdeev, 2002) and other methods (Lu & Liu, 2002). The quenching of the singlet states of flavins is mostly connected with their interactions with the aromatic compounds, aminoacids and peptides (Ulbrych & Siódmiak, 1982; Mieloszyk *et al*., 1989; Muñoz *et al.*, 1995) and with the formation of flavin dimers in solutions (Grajek *et al*.: 1990, 1992; Grajek, Żurkowsk,& Kuśba, 2005; Grajek, 2007; Bojarski P., Grajek, Żurkowska, Kukliński, Smyk & Drabent, 1999; Bojarski P, Kułak, Grajek, Żurkowska, Kamińska, Kukliński & Bojarski C., 2003).

Many biological compounds (Gruszecki, Gagoś & Hereć, 2003a; Gruszecki, Gagoś, Hereć& Kernen, 2003b; Gruszecki, Gagoś & Kernen, 2002; Sujak, Okulski & Gruszecki 2000; Frąckowiak, Zelent, Malak, Planner, Cegielski, Munger & Leblanc, 1994; Frąckowiak, Dudkowiak, Ptak, Malak, Gryczyński & Zelent, 1998; Nowicki, Susla, Planner & Frąckowiak 1999) like proteins, enzymes, organic dyes and synthetic compounds (e.g. rhodamines) form dimers. Paying attention to many different photoreceptors leads to the conclusion that many photobiological processes take place with the contribution of dimers. For example, the dimers of chlorophyll appear first of all in both photosynthetic centers, both in PS1 and in PS2. Phytochrome and carotenoids act also as dimers in the photoreception phenomena. It follows from these facts that dimers can play a role of incident light traps in many phenomena occurring in living organisms.

INVESTIGATIONS OF FLAVINS DIMERIZATION. FLAVINS DIMERS IN PHOTORECEPTION SYSTEMS.

The studies of flavins dimerization started in 1956 when Beinert suggested based on the concentration changes of absorption at 900 nm the formation of a charge-transfer (CT) complex between the oxidized and reduced form of FMN (FMN-$FMNH_2$) or the formation of a semiquinone dimer. Similarly, Gibson *et al* (Gibson, Massey & Atherton, 1962) studied the mixture of FMN with $FMNH_2$. By recording absorption spectra with the maxima at 570 nm and 900 nm they concluded the formation of dimers of the reduced form of $FMNH_2$ flavin and CT complexes. These have not been, however, the studies of oxidized forms of FMN flavin characterized by absorption maxima at 445 nm and 370 nm. The mentioned authors claimed that FMN dimerization does not affect the absorption maximum at 445 nm. Almost no further investigations have been undertaken concerning FMN dimerization process until the eighties. Meanwhile, only several authors performed relevant studies: Müller *et al* (1973) observed the temperature changes in absorption spectra due to flavins (and flavins derivatives) dimers. The existence of flavins dimers has also been proposed by Harders *et al.* (1974), Visser *et al.* (1974) and Heelis *et al.* (1978). Song *et al* (1972) based on the phosphorescence excitation spectra at T=77 K suggested extremely weak fluorescence of riboflavin (and its derivative) dimer. The mentioned studies have not been, however, oriented towards the determination of dimerization constant and

investigation of dimerization process (which is the case of many other aggregating dyes).

In 1968 Sarma *et al*.(Sarma, Dannies & Kaplan, 1968) followed by Kainosho & Kyogoku. (1972) based on NMR studies proposed the models of FMN dimer, in which FMN monomers were aligned "face to face" one above the other and and slightly shifted. These models differ by the shifts of isoalloxazine rings.

Typical studies of FMN dimerization process have been carried out for FMN in water and glycerol–water solution in 1984 and later (Grajek *et al*.: 1984, 1986; 2001). In these works the effect of dimerization on the absorption spectrum of flavins has been studied. The spectra of pure FMN monomer and dimer as well as the dimerization constant in water have been determined (K=118.0 M^{-1} at T=298.2 K, water) and (K=20.5 M^{-1} at T=298.2 K, glycerol-water mixture, viscosity 056 P) (Grajek *et al*. 1984). Strong dependence of absorption FMN spectrum in water on temperature has been found. The dimerization constant changed from K=330 M^{-1} at T=275.2K to K=26 M^{-1} at T=339.7K (Grajek, Żurkowska, Drabent & Bojarski, 2001). The studies of FMN dimerization have been performed also in a rigid PVA matrix (K=11.6 M^{-1}) (Grajek *et al*., 1998, Grajek 2007), where FMN dimers occurred durable and where the temperature increase even up to 338 K did not lead to their decomposition.

The FMN dimer structure has been thoroughly defined in water and in a viscous solution (Grajek et al. 1986) as well as in rigid PVA (Grajek *et al*., 1998; Grajek 2007) based on the spectroscopic studies and Kasha theory. It has been found that FMN dimers form "sandwiches", in which two monomers are aligned "face to face" one above the other at a distance R= 3.5 ± 0.3 Å (in water) and R=3.2 ± 0.3 Å (in PVA) in such a way that the ribityl chains are in opposite directions. FMN monomers are slightly mutually twisted so that the CH_3 groups do not form a sterical obstacle. The transition moments responsible for the first transition in the absorption band in water form an angle $\alpha_I = 71 \pm 4°$, whereas the moments responsible for the second transition form an angle $\alpha_{II} = 0 \pm 4°$. For FMN dimer in PVA the following respective values have been obtained: $\alpha_I = 75^o \pm 4^o$, $\alpha_{II} = 0^o \pm 4^o$. The hydrophobic interactions are responsible for the binding of izoalloxazine rings. It has been found that the van der Waals interactions bind izoalloxazine rings of flavins through the interaction of π-electronic system while four water molecules stabilize this dimer through the hydrogen bonds (Grajek *et al*., 1986). The obtained structure of FMN dimer is in agreement with the analysis of chemical interactions , with the transition moments directions determined by Johansson *et al.* (1979) and with the NMR studies. FMN dimer structure has been confirmed by the molecular modeling calculations (Smyk & Grajek 2001).

Such a structure of the FMN dimer has been obtained as a result of the numerical decomposition of dimer absorption spectrum into simpler components. One can see from pure dimer spectrum (Fig.3B) that there appears a shoulder at about 480 nm which results from the Davidov splitting of the first singlet excited state into two levels: H and J corresponding to the $S_0 \rightarrow S_1$ in the monomer. Hence, two bands H and J appear in the dimer spectrum. The comparison between the dimer spectrum and the action spectra for the chloroplast (Fig.3C and Fig.3D) (Zurzycki 1962) one can note greater similarity of action spectra to the FMN dimer absorption spectrum than to that of monomer.

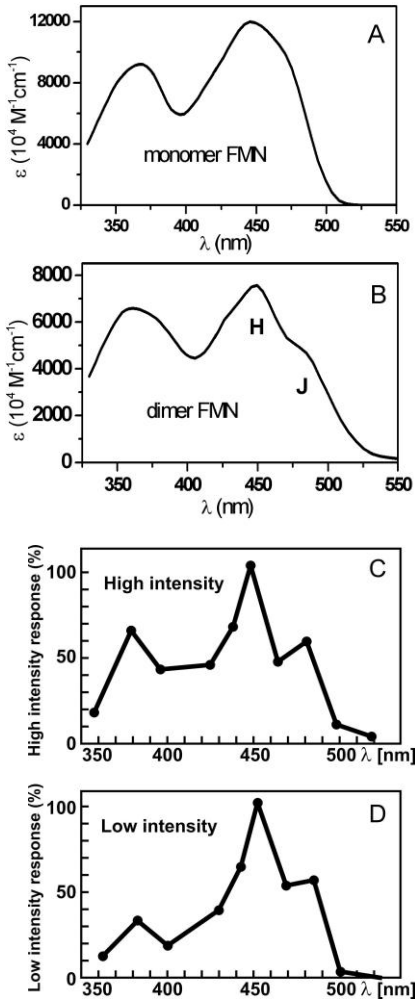

Fig.3. The comparison between FMN monomer and dimer absorption spectra with the action spectra for chloroplast orientations: A – FMN monomer absorption; B – FMN dimer absorption spectrum with the indicated H and J bands location corresponding to $S_0 \rightarrow S_1$ transition; C – Action spectra for chloroplast orientation in *Lemna trisulca* – high intensity, D – low intensity. (Fig. 3C and 3D based on Fig.33 by Zurzycki, 1962).

This is quite interesting having in mind prolific recent communications on FMN dimers existence in biological organisms and photoreceptors. Recently, Muralidhara and Wittung-Stafshede (2003) confirmed based on the calorimetric studies of isothermic titration, NMR as well as absorption and fluorescence spectroscopy the structure of FMN dimer obtained by Grajek *et al*. (1986) by discovering the FMN dimer in a flavodoxin protein, the co-factor of which is FMN. They proved the existence of a "stacking" FMN dimers bound with the apoflavodoxin *Deslfovibrio desulfuricans*. The FMN dimer interacts with three protein loops in such a way that one FMN monomer from the dimer forms a sandwich with the tryptophan Trp 60 and the second one with the tyrosine Tyr 68. This has been the first proof of FMN dimer existence in the biological system. Also Bieger *et al*. (Bieger, Essen & Oesterhelt, 2003) based on crystallographic studies proved the existence of six riboflavin dimers in dodecine, a flavoprotein from *Halobacterium salinarium.*

However, no studies on flavin photoreceptors have been performed until now to find out what is actually a flavin photoreceptor: monomer or dimer of a flavin? In the literature the dimerization of flavins in biological systems still remains an open question. The possibility of a flavin dimers formation in biological systems is possible due to close proximity of flavin monomers as reported in the literature.

Crosson and Moffat (2001) considered the possibility of dimerization between LOV1 and LOV2 domains In a single phototropin molecule. Salamon *et al.* (Salomon, Lempert & Rüdiger, 2004) proved the formation of dimers from phototropin fragments with flavin domains LOV1 and LOV2 (without the kinaze part) and have shown that LOV1 domains form dimers in a solution, while LOV2 does not exhibit such a property. The conclusion has been drawn that the phototropin is a dimer and that the LOV1 domain is probably responsible for the dimerization. Nakasako *et al*. (Nakasako, Matsuoka, Zikihara & Tokutomi, 2005) studied the dimeric association of photoreceptors regulating the flowering of *Arabidopsis* and have shown the antiparallel alignment of LOV domains with FMN in a dimer. Also Kitizing *et al*. (Kitizing K, Fitzpatrick, Wilken Sawa, Bourenkov, Macheroux & Clausen, 2005) have shown based on crystallographic measurements of flavoproteins YqjM that this is a tetrameric enzyme organized as a dimer of two active dimers. Mutual alignment of FMN monomers in this tetramer has been defined.

It must be herein underlined that the action spectra of chloroplast movement (Gabryś 1985; Gabryś 1986; Gabryś *et al*., 1985), photaction of *Euglena* and phototropism of circadian rhythm in *Neurospora*, the action spectra of b-cytochrome photoreduction in *Neurospora* (Ninnemann 1980; Galland & Lipson 1985) as well as the absorption spectra of cryptochrome Cry1 (Whitelam, 1995), LOV domains (Salomon *et al* 2000; Swartz *et al*. 2001; Kasahara *et al.* 2002), FAD binding domains in AppA (Masuda *et al.* 2002, Kraft *et al.* 2003) and others shown in Fig.2 are more similar to flavin dimers than to monomers (compare.Fig.2 with Fig.3A and Fig.3B). The characteristic shoulder in the action spectrum at about 480 nm, which exists also in the flavin dimer absorption spectrum (Grajek *et al*. 1984, 1986, 2001) can indeed suggest that the flavin dimer plays a role of a photoreceptor (despite flavoproteins also possess weakly visible shoulder). Nevertheless, based only on these spectra it would be difficult to undoubtedly state that flavin dimers are photoreceptors in these processes. This is an open problem for further studies of flavins and excitation energy transfer process between them.

Flavins in biological systems appear in rigid environment: peptide structures or membranes so it is justified to study nonradiative excitation energy transfer in FMN monomer – dimer system and the spectroscopic properties of dimers in model rigid media (PVA films). FMN dimers in a rigid medium exhibit their own fluorescence (Grajek *et al*. 1998; Grajek 2007; Grajek *et al*. 2007a), which yields the possibility to study energy transfer acts originating from dimers (Bojarski P *et al* 2003; Grajek 2007). It has not been possible until now to achieve this for other dyes as at high concentrations they usually form higher order aggregates. FMN, however, does not form higher order aggregates due to sterical effects (Grajek *et al.*, 2001; Grajek 2003; Grajek, Gryczyński I, Bojarski, Gryczyński Z, Bharill & Kułak, 2007b). Also nonradiative excitation transfer between the monomers and dimers of FMN as well as energy migration between FMN monomers have been studied in liquid solutions (Grajek *et al.* 1990, 1992, 2005). It occured that in liquid solutions FMN dimers are perfect traps for excitation energy for the exciting light and excitation energy transferred to them from monomers. Qualitative and quantitative investigations of energy transport for FMN have been performed using Bojarski theory (Bojarski & Domsta, 1971) of multistep excitation energy transport (MEET). It has been found that the energy transfer to the dimer is preceded by energy migration between FMN monomers (Grajek, 1990; 1992). Good correspondence between the theoretical results of concentration quenching of fluorescence η/η_0 (Grajek *et al*., 1990) and concentration depolarization r/r_0 (Grajek, Żurkowska, Bojarski & Drabent, 1992) with respective experimental data in glycerol-water solutions confirmed multistep excitation energy transport. Experimental studies of FMN quantum yield in water with the application of MEET model (Grajek, Żurkowska & Kuśba, 2005) have shown an important role of material diffusion in accelerating energy transport and making it more

efficient. The agreement between the theoretical results of η/η_0 of the model extended by Kusba to material diffusion effect (Grajek, *et al.,* 2005) proved that the relative contribution of the diffusion accelerated nonradiative energy transfer to the total drop of the quantum yield can be even higher than 70%. The analysis has also shown that it is necessary to take into account an additional channel of deactivation of the excitation energy transfer, i.e., its partial degradation during the successive steps of migration between monomers. The probability of such a process can be as large as 0.6 – 0.7 . However, for FMN in rigid PVA matrix it has been shown that FMN dimers are fluorescent (Grajek *et.al.,* 1998; Grajek 2007; Grajek, Liwo, Wiczk & Żurkowska, 2007a) and able to transfer energy back to monomers (reverse energy transfer). Indeed, experimental and theoretical studies of energy transport between FMN monomers in rigid matrices have shown the presence of reverse energy transfer from dimers to monomers and the effect of energy migration between FMN dimers. Both hopping model of reverse energy transport (Bojarski C, 1984; Grajek 2007) and self-consistent diagrammatic model (Kułak & Bojarski, 1995; Bojarski P. *et al*., 2003) have been applied.

Moreover, these studies have shown the role of excitation energy sink which takes place between the equilibrated FMN centers M_1, M_2, M_3, which occurs as a result of inhomogeneous broadening of energy levels

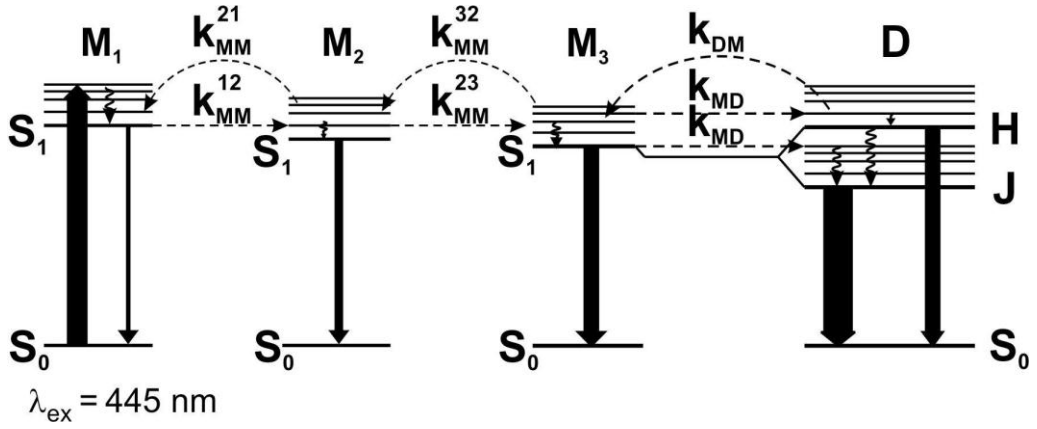

Fig.4. Diagrams of monomer M and dimer D energetic levels for FMN. Any possible ways of energy transfer between FMN monomers and dimers in rigid PVA system are shown. M_1, M_2, M_3 - denote monomeric equilibrated centers with decreasing energies of 0-0. k_{MM}^{12}, k_{MM}^{23} - rate constants for energy migration; k_{MM}^{21}, k_{MM}^{32} - rate constants for remigration; $M^* \rightarrow D$ – directional energy transfer with the rate constant k_{MD} and reverse energy transfer $D^* \rightarrow M$ with the rate constant k_{DM}.

(IBEL). Figure 4 visualizes excitation energy migration among these centers, the directional excitation energy sink from monomers to the dimers $M^* \rightarrow D$ and the reverse transfer $D^* \rightarrow M$ (from Żurkowska, Grajek H.& Bojarski, 1996; Grajek *et al.* 1998; Grajek 2007). This example shows the degree of development of original quantum- mechanical Förster model of single-step energy transfer.Energy transport processes in biological systems may be even more complex (Frąckowiak & Fiksiński, 1976). By now it has not been shown how exactly energy transfer with the participation of flavins takes place in biological systems. In (Żurkowska *et.al*., 1996; Grajek et al. 1998; Grajek 2007) it has been shown that for FMN, IBEL extremely strongly affects the tracks of excitation sink. It is an open question if and how biological species would "choose" the most effective tracks for energy transport as already observed in ordinary in vitro FMN rigid systems.

CONCLUSIONS

Flavins as biological molecules are inspiring to investigate not only for biologists and biochemists but also for physicists. These molecules take part in excitation energy transport effectively. Therefore the number of papers dealing with this subject gradually increases. Flavins participating in the photoreception phenomena transfer energy to the reaction center. It is expected that new valuable information on energy transfer range will be obtained with modern experimental techniques (ultra fast measurements of

fluorescence decays with time-correlated single photon counting method, up-conversion technique and time resolved emission spectra measurements) when correlated with the theory of nonradiative excitation energy transfer. Such studies are planned among others biochemical reaction. 2) global quantum yields of excitation energy transfer from monomers to dimers and the analogous yields of energy transfer from monomers of particular orders of excitation; 3) global average lifetimes of monomers in the excited state and average residence time of excitation energy on monomers of particular orders of excitation. This will allow for more profound and quantitative investigation of excitation transfer between FMN molecules. Especially important are herein the investigations in the presence of FMN dimers. The use of time resolved techniques will allow that by immediate observations of time evolution of the monomer and dimer emission spectra of FMN it will be possible to confirm existence of fluorescence originating separately from the excited dimer states H and J, and observe existence of the long-wavelength dimer centers. There is a lack of reports in the literature, related to such type of investigations.

However, the confirmation of hypothesis suggested in this work that in some photoreception phenomena the flavins dimers can play a role of photoreceptors requires further investigations performed directly with the biological material.

ACKNOWLEDGMENTS

The author would like to thank Professor Halina Gabryś from the Cracow Jagiellonian University for the valuable discussions on biological aspects of this work.

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in our group. They will enable to obtain 1) the mean number of excitation jumps since the moment of absorption to the moment of emission in the form of quantum of light or conversion into heat or its loss in a

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