

CONFORMATION OF FMN DIMER OBTAINED BY MOLECULAR MODELLING METHODS

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The structure of flavomononucleotide (FMN) is obtained by molecular modelling methods. At the first stage the geometry of FMN molecule was optimized. The maximal dimension of FMN monomer was obtained equal $\delta=15.82\text{\AA}$. Then, the structure of dimer both in vacuum and in the vicinity of 302 water molecules was calculated in a periodic box. HyperChem software (version 5.02) was used to obtain the stacking structure of the dimer. We found that the isoalloxazine rings are almost parallel, shifted one to another by about 0.58\AA towards the short axis of the ring and slightly twisted one to another at an angle $\alpha=3.5^\circ$. For the average distance between isoalloxazine rings the value of 3.57\AA has been obtained in vacuum compared to that of 3.70\AA in the vicinity of water.

INTRODUCTION

Flavomononucleotide (FMN) is a photoreceptor and coenzyme in many biological reactions taking place in living organisms (Haupt & Wagner, 1984). There are well known papers (Ninnenmann, 1980; Walczak, Gabryś & Haupt, 1984; Schmidt, 1980; Quinones & Aparicio, 1990), the authors of which prove that flavin can play a role of a photoreceptor in protoreception phenomena. FMN may appear as a monomer or as a dimer (Grajek, Żurkowska, Drabent & Bojarski, 1986; Grajek, Drabent, Żurkowska & Bojarski, 1984; Grajek, Żurkowska, Drabent & Bojarski, in press).

Investigations of dimerization process are important in view of nonradiative excitation energy transport in the photoreception processes in which flavins take part. Depending on its environment FMN dimer can either be a perfect trap for excitation energy (in aqueous solutions) (Grajek, Żurkowska, Bojarski & Drabent, 1990) or it can fluoresce in rigid systems like polyvinyl alcohol (PVA) films (Grajek, Żurkowska, Bojarski, Kukliński, Smyk, Drabent & Bojarski, 1998). This dimer fluorescence affects, of course, the mechanism of energy transport. Usually, dimer fluorescence is weak and temperature dependent (Bojarski, Grajek, Żurkowska & Smyk, 1998; Bojarski, Grajek, Żurkowska, Kukliński & Smyk, 1999). The structure of the dimer, i.e., mutual orientation of monomers in a dimer affects the spectroscopic properties of FMN and the mechanism of energy transport.

Previously (Grajek, Żurkowska, Drabent & Bojarski, 1986), structure of FMN dimer has been proposed based on a comparison between concentration dependence of absorption spectra and Ka-

sha theory. In the present work we would like to present the FMN structure obtained based on molecular modelling methods.

METHODS

FMN monomer structure was obtained using the MM+ method based on the classical force field. We used the Polak-Riebiere computing method. After this preliminary optimization of FMN molecule, further calculations were performed based on the semiempirical quantum PM3 method with the following parameters: total charge = 0, spin multiplicity = 1, spin pairing – RHF, state -lowest, configuration interaction -single excited.

The geometry of FMN dimer was optimized in a similar way first in vacuum and then, in the vicinity of 302 molecules of water. Calculations were carried out using HyperChem 5.02 software which includes both computational methods based on classical mechanics and quantum mechanics. During calculations we used Pentium III 500 MHz computer with 256 MB RAM.

RESULTS OF CALCULATIONS AND DISCUSSION

Structure of FMN monomer

The structural formula of FMN is presented in Fig.1. Fig.2 shows the configuration of FMN monomer obtained as a result of calculations performed. Planar configuration of isoalloxazine FMN ring has been obtained. Our calculations showed a slight deviation, about 15° , of ribitol chain from

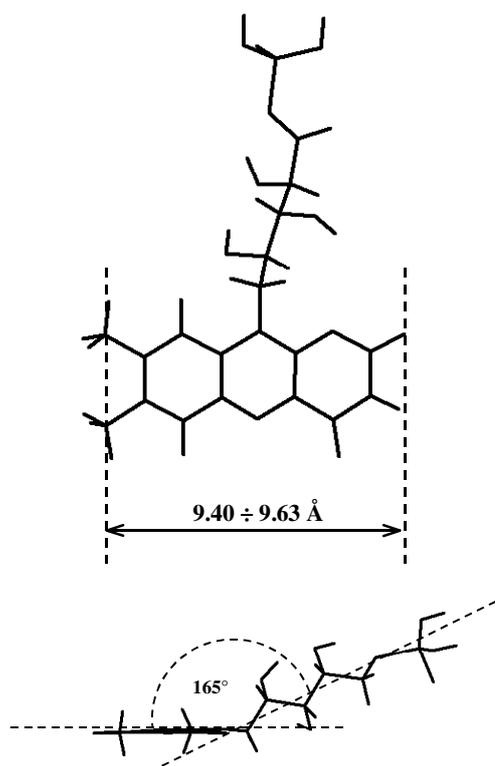


Fig.2. Optimized configuration of FMN molecule.

the plane of isoalloxazine ring. It results from the fact that the axis linking N(5) and N(10) atoms of the ring makes an angle of 165° with the straight line connecting the atoms N(10) and that of phosphor P at the ribitol chain.

The selected dimensions of FMN monomer were as follows:

The distance, δ , in the direction of main axis of isoalloxazine ring:

$$\begin{aligned}\delta[\text{CH}_3(\text{C}(8)), \text{O}(\text{C}(2))] &= 9.63 \text{ \AA} \\ \delta[\text{CH}_3(\text{C}(7)), \text{H}(\text{N}(3))] &= 9.40 \text{ \AA}\end{aligned}$$

The distance, δ , in the direction of diagonal of the ring:

$$\delta(\text{CH}_3(\text{C}(7)), \text{O}(\text{C}(2))) = 9.99 \text{ \AA}$$

The distance, δ , in the direction of axis perpendicular to the main axis of the molecule – the length of molecule including ribitol chain:

$$\delta[\text{N}(5), \text{P}_{\text{ribitol}}] = 11.7 \text{ \AA}.$$

The maximal dimension of FMN molecule, ie the dimension between the hydrogen atom of methyl

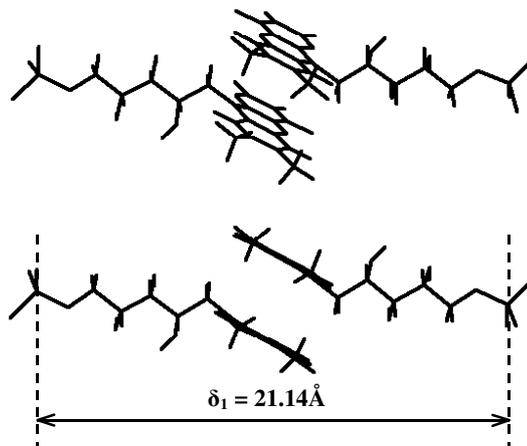


Fig.3. Three projections of FMN dimer structure in vacuum.

group at C(7) and the P oxygen of the ribitol chain:

$$\delta[\text{H}(\text{CH}_3(\text{C}(7))), \text{PO}^-_{\text{ribitol}}] = 15.82 \text{ \AA}.$$

Schmidt (1980) and Tanaka (1967) obtained similar dimensions of isoalloxazine ring.

FMN dimer structure

To obtain the structure of FMN dimer two FMN molecules were brought close together. Then the repulsive forces were allowed to act by employing the classical field MM+ and then PM3 field. As a result the structure of a dimer has been obtained, in which isoalloxazine rings were located parallel one to another and shifted 0.58 \AA in the direction of short axis of the ring. They were also slightly twisted with an angle $\alpha=3.5^\circ$. The distances between particular atoms of FMN monomers in a dimer located opposite one to another expressed in \AA are as follows:

$$\begin{aligned}\delta[\text{N}(5), \text{N}(10)] &= 3.46 \text{ \AA} \text{ first pair, } \delta[\text{N}(5), \text{N}(10)] = 3.45 \text{ \AA} \text{ second pair} \\ \delta[\text{C}(4), \text{N}(1)] &= 3.59 \text{ \AA} \text{ first pair, } \delta[\text{C}(4), \text{N}(1)] = 3.54 \text{ \AA} \text{ second pair} \\ \delta[\text{C}(6), \text{C}(9)] &= 3.70 \text{ \AA} \text{ first pair, } \delta[\text{C}(6), \text{C}(9)] = 3.70 \text{ \AA} \text{ second pair}\end{aligned}$$

The mean distance calculated between isoalloxazine rings in vacuum was 3.57 \AA .

The FMN dimer dimensions have also been calculated: length along the axis including the ribitol chains $\delta_1=21.14 \text{ \AA}$ and length along the main axis of isoalloxazine rings $\delta_2=9.76 \text{ \AA}$.

Fig.3 shows the structure of FMN dimer in three projections. A small shift of parallel placed isoal-

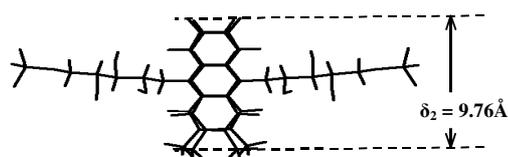
loxazine rings as well as mutual twist of rings including CH_3 groups can be seen. This is because the location of methyl groups one above another would be energetically disadvantageous.

Grajek, Żurkowska, Drabent & Bojarski, (1986) obtained the distance between monomer units in the dimer, $R=3.5 \pm 0.3 \text{ \AA}$, which is in accordance with the smallest distance allowed by the Van der Waals interactions. They showed that apart from Van der Waals interactions, water molecules have a great effect on the stabilization of this dimer by forming hydrogen bonds between FMN molecules. Thus water, acting as a proton donor, links the N(1) of the first FMN molecule with the C(4) oxygen of the second molecule. The molecule also links the N(3) hydrogen atom of one molecule (the water molecule acting as a proton acceptor) with the C(2) oxygen of second molecule (where H_2O acts as a proton donor).

The FMN dimer structure obtained by molecular computation method is therefore in accordance with the results obtained previously (Grajek, Żurkowska, Drabent & Bojarski, 1986) and with the results of FMN dimer structure obtained with the help of NMR spectroscopy (Kainasho & Kyogoku, 1972).

After calculating the FMN dimer structure in vacuum further computations of the dimer structure, taking into account the presence of 302 water molecules have been carried out. It was assumed that water molecules are placed in a periodic box from which they can not escape. The obtained FMN dimer structure is shown in Fig. 4. The geometry of the dimer has not changed significantly. Only the distance between particular atoms of isoalloxazine rings increased a little which is illustrated by following data:

$$\begin{aligned} \delta[\text{N}(5), \text{N}(10)] &= 3,47 \text{ \AA} \text{ first pair} & \delta[\text{N}(5), \text{N}(10)] \\ &= 3.50 \text{ \AA} \text{ second pair} \end{aligned}$$



$$\begin{aligned} \delta[\text{C}(4), \text{N}(1)] &= 3,85 \text{ \AA} \text{ first pair} & \delta[\text{C}(4), \text{N}(1)] \\ &= 4.09 \text{ \AA} \text{ second pair} \\ \delta[\text{C}(6), \text{C}(9)] &= 3,67 \text{ \AA} \text{ first pair} & \delta[\text{C}(6), \text{C}(9)] \\ &= 3.63 \text{ \AA} \text{ second pair} \end{aligned}$$

The mean distance between the isoalloxazine rings in the vicinity of water occurred to be 3.70 \AA .

From the calculations performed it follows that the methods applied allow to make visible additional details of molecular structures. The method applied showed slight mutual shift of FMN isoalloxazine rings and unequal distances between particular atoms located one opposite to another as well as it explained that the twisting of long axes of monomers in the dimer is caused mainly by the twisting of rings containing CH_3 groups.

Both in water and in vacuum the same structure of FMN dimer has been obtained with a slight difference in mutual distances between isoalloxazine rings. In the absence of water molecules around those of FMN the mean distance between isoalloxazine rings in the dimer was shorter than that in water. In vacuum the FMN monomers are bound together due to Van der Waals interactions between isoalloxazine rings which was confirmed by our calculations. The Van der Waals 'radius' of half of the π -electron aromatic system is equal to approx. 1.7 \AA (Pauling, 1962). The mean distance calculated between units in the dimer in vacuum 3.57 \AA , is in accordance with the smallest distance allowed by the Van der Waals interaction.

However, for FMN molecules surrounded by water, the decisive role in the stabilization of FMN

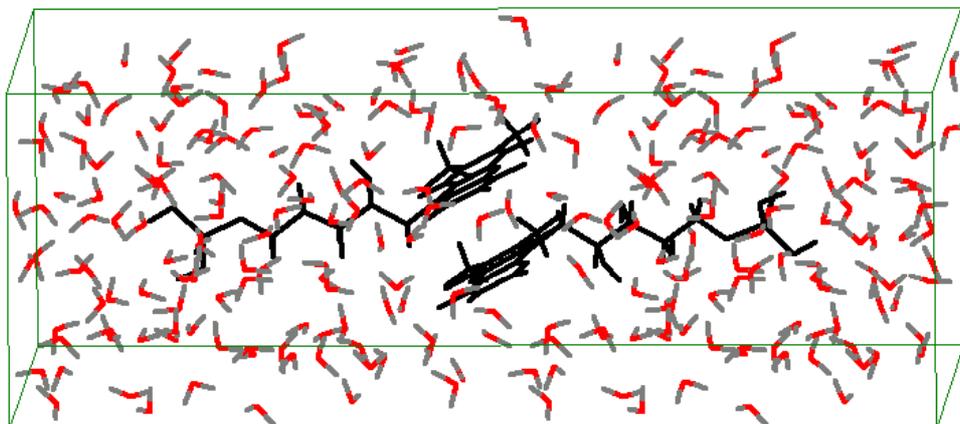


Fig.4. FMN dimer structure in the vicinity of water molecules.

dimer is played by water molecules through the formation of hydrogen bonds between isoalloxazine rings. Four water molecules take part in isoalloxazine rings binding (Grajek, Żurkowska, Drabent & Bojarski, 1986). The value of enthalpy change for FMN dimerization process was calculated based on temperature dependence of FMN absorption spectra in water (Grajek, Żurkowska, Drabent & Bojarski, 2001). This value, $\Delta H = -39.8$ kJ/mol, occurred to be in accordance with the value of hydrogen bond energy (Weeny, 1979). Taking into account the dimensions of water molecule and the length of hydrogen bonds (Sobczyk, 1969) one can conclude that the distance between the monomers bound through water must be slightly longer than in vacuum, which is suggested by the calculated mean distance 3.70\AA between FMN monomers in the dimer in water.

Flavins in living organisms appear as flavoproteids in hydrophobic environment — a protein pocket. Flavins taking part in photoreception phenomena are located in a cell membrane of chloroplasts. Model studies of flavins in membranes have shown that flavins are either built into a lipid membrane or they can be found at the membrane-water interface (Frehland & Trissl, 1975). Hence, it was justified to perform model calculations of the dimer structure in aqueous phase and in vacuum. Proof by spectroscopic methods that FMN monomers form dimers (Grajek, Drabent, Żurkowska & Bojarski, 1984; Grajek, Żurkowska, Drabent & Bojarski, 1986) as well as confirmation of dimer structure by molecular modelling methods may deliver important information for the analysis of photoreception processes in living organisms, in which flavins take part. This conclusion is strongly supported by the fact that dimers in aqueous environment are traps for exciting light (Grajek, Żurkowska, Bojarski & Drabent, 1990) as well as by the similarity of chloroplast action spectra to those of FMN dimers but not monomers (Ninmann, 1980).

REFERENCE

- Bojarski P., Grajek H., Żurkowska G., Kukliński B., Smyk B. & Drabent R. (1999). Excitation energy transport in concentrated system of flavomononucleotide in polyvinyl alcohol films. *J. Fluorescence*, **9**, 391-396.
- Bojarski P., Grajek H., Żurkowska G. & Smyk B. (1998). Quantum yield of flavins in monomer – fluorescent dimer system. [In:] Holick M. F. & Jung E. G. (Eds.) *Biologic effect of light* (pp. 475-478). Boston/London/Dordrecht: Kluwer Academic Publishers.
- Frehland E. & Trissl H.W., (1975). Fluorescence polarization in a planar array of pigment molecules: theoretical treatment and application to flavins incorporated into artificial membranes. *J. Membrane Biol.*, **21**, 147-183.
- Grajek H., Żurkowska G., Drabent R. & Bojarski C. (in press). The effect of temperature and concentration on the aggregation of flavomononucleotide (FMN) in aqueous solutions. *Asian J. Spectroscopy* –.
- Grajek H., Żurkowska G., Bojarski P., Kukliński B., Smyk B., Drabent R. & Bojarski C. (1998). Spectroscopic manifestations of flavomononucleotide dimers in polyvinyl alcohol films. *Biochim. Biophys. Acta*, **1384**, 253-267.
- Grajek H., Żurkowska G., Bojarski C. & Drabent R. (1990). Investigation on the fluorescence concentration quenching of flavomononucleotide in glycerine-water solution. *Photochem. Photobiol.*, **51**, 565-571.
- Grajek H., Żurkowska G., Drabent R. & Bojarski C. (1986). The structure of the flavomononucleotide dimer. *Biochim. Biophys. Acta*, **881**, 241-247.
- Grajek H., Drabent R., Żurkowska G. & Bojarski C. (1984). Absorption of flavin dimers. *Biochim. Biophys. Acta*, **801**, 456-460.
- Haupt W. G. & Wagner G. (1984). Chloroplast movement. [In:] Colombetti G. & Lenci F. (Eds.) *Membrane and sensory transduction* (pp. 331-375). New York: Plenum Press.
- Kainosho M. & Kyogoku Y. (1972). High-resolution proton and phosphorus nuclear magnetic resonance spectra of flavin – adenine dinucleotide and its coformation in aqueous solution. *Biochemistry*, **11**, 741-752.
- McWeeny R. (1979). *Coulson Valence*. Oxford: Oxford University Press.
- Ninmann H. (1980). Blue light photoreceptors. *Bio Science*, **30**, 166-170.
- Quiñones M. A. & Aparicio P. J. (1990). Flavin type action spectrum of nitrate utilization by *Monoraphidium Brauni*. *Photochem. Photobiol.*, **51**, 689-692.
- Pauling L. (1962). *Die Natur der Chemischen binary*. Weinheim: Verlag Chemie.
- Shmidt W. (1980). Artificial Flavin / Membrane systems; a possible model for physiological blue light action. [In:] Senger H. (Ed.) *The Blue Light Syndrome* (pp. 212-220). Berlin-Heidelberg: Springer-Verlag.
- Sobczyk L. (1969). *Wiązania wodorowe*. PWN, Warszawa.
- Tanaka N., Ashida T., Sasada Y., Kakudo M. (1967). The crystal structure of riboflavin hydrobromide monohydrate. *Bull. Chem. Soc. Jap.*, **40**, 1739-1739.
- Walczak T., Gabrys H. & Haupt W. (1984). Flavin mediated weak light chloroplast movement in *Mougeotia*. [In:] Senger H. (Ed.) *Blue light effect in biological systems* (pp. 212-220). Berlin-Heidelberg: Springer-Verlag.