Current Topics in Biophysics 2000, 24(1), 3-13

BIOPHYSICS IN TECHNOLOGY AND MEDICINE

DANUTA FRĄCKOWIAK, JACEK GOC, ANETA WASZKOWIAK

Institute of Physics, Poznań University of Technology, Piotrowo 3, 60-965 Poznań, Poland

In this review following topics are shortly characterised: 1. conversion of light energy into the electric energy using the system containing elements of photosynthetic organisms, 2. production of hydrogen-ecological fuel, produced by some bacteria mutants,3. photodynamic cancer therapy, predominantly the use of dye in oncology.

First of all one should establish what biophysics is. Does the biologist studying the tissue under the best electronic microscope automatically becomes the biophysicist? If he only describes the structure of the tissue, that means — he isn't. He is still the biologist using specialised physical equipment. As soon as he reaches for the atom force microscope and will base the interpretation of his macromolecule observation on the theory of intermolecular interaction, then he can be called a Biophysicist. One may say generally that Biophysicist uses the theory of physics to explain processes which take place in organisms, if it wasn't for the fact that nowadays the interpretation of theory of physics takes into account only very sophisticated, formalised mathematical reasoning, whereas in biophysics we often reach for the already well known, very basic principles of physics. Therefore Biophysicist needs to be a biologist as well as a physicist at the same time. It does not mean at all that he has to have two degrees or graduate from The Faculty of Biophysics but has to be able to deal with two subjects simultaneously at once.

In the past people could cope to perfection in many fields. Copernicus for example was not only an astronomer but also a physician and an economist. Goethe did not only describe "The Sufferings of the Young Werter" and ups and downs of Faust but also eagerly fought in one of his thick publication against Newton's views - that the white light can be obtained by the overlapping of multicolour light beams. He was far better at biology and very inquisitively analysed the similarity of various organisms. Such results at a later stage help Darwin to form the theory of evolution. Goethe, definitely was not the Biophysicist – his studies in physics and biology were performed completely separately. We have learned of his activities in science pretty recently from a very interesting book by A. K. Wróblewski "Scientists in Anecdote" (Wróblewski, 1999).

Due to a very vast number of information and the development of research methods in various fields there used to be a trend for a very narrow specialisation. Then, such professions as a physicist, chemist or a biologist existed completely separately, but now is the tendency to carry studies on the boarders of these three domains. It was not only in order to avoid a very narrow specialisation where one knows "everything about nothing" but also to get advantage of numerous different applications, based on achievements gained on the boarder of various fields.

In technics the results of physical research were utilised from as long as one can remember. Analysing the nature, physicists did not think of a practical applications of their findings. Once someone asked professor A. Kastler if he had ever thought of laser and its use when he was working on the optical pumping. He very sincerely stated that what made him happy was the possibility of obtaining high population of excited states, but at that stage he never thought of the practical use of such effect. Only further years introduced the invention of laser and the numerous applications of lasers in physics, technology and medicine.

It is very similar with Biophysics: it is very difficult to predict as to what one can apply one's research as there is more and more usage. We would like to talk only about a few of them – the ones which we dealt with in our laboratory, such as:

(i) conversion of light energy into the electric energy using the system containing elements of photosynthetic organisms,

(ii) production of hydrogen ecological fuel, produced by some bacteria mutants,

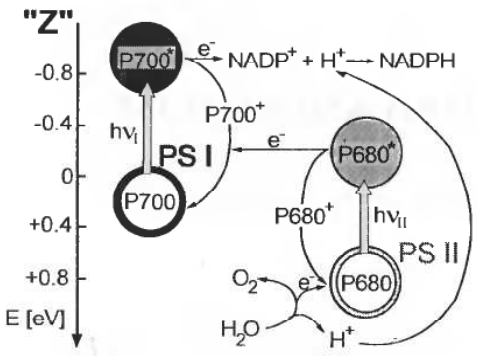

Fig. 1 Simplified scheme of photosynthesis „Z" process of algae and green plants (PS I i PS II – photosystems first and second; P700, P690 – reaction centres of PS I and II.

(iii) photodynamic cancer therapy – or generally speaking – the use of dye in oncology.

It is worth reminding ourselves the process of photosynthesis. We all know that in the autotrophs such as higher plants, thanks to the absorption of light and the use of carbon dioxide as well as due to decomposition of water, carbohydrates (CH_2O) are synthesized according to the following reaction:

$$nCO_2 + nH_2O \xrightarrow{h\nu} nO_2 + (CH_2O)_n \qquad (1)$$

Every day we witness the effect of plants refreshing air due to carbon dioxide being absorbed and oxygen being emitted, both of which happen in a rather complex, strongly anisotropic photosynthetic apparatus of organisms. There are several different pigments responsible for absorbing radiant energy in photosynthetic organisms, with absorption spectra matching the spectrum of the light reaching the organisms.

In order to get one "brick" of carbohydrate CH_2O one needs eight quantum of light; four for each photochemical reaction taking place in two reaction centres. For the photosynthesis to take place, both centres have to co-operate with one another. Some "dark" reactions also need to take place which we will not discuss but which are marked in Fig. 1. The process of light absorption and excitation energy transfer to reaction centres takes place in pigment-protein complexes. These pigmented macromolecules called antenna complexes are arranged in such a way so they force migration of the excitation energy in the privileged direction to the reaction centre. It happens thanks to the way the dye complex with lower excitation energy being placed closer to reaction centres. As we know it is much easier to diffuse part of the energy into the heat than getting it back. The transfer of the energy takes place without emission and absorption of quantum - only excitation energy gets through. Mechanism of transferring such energy depends on the structure of photosynthetic apparatus. If pigments are attached to large macromolecules and thanks to this are quite far away from each other ("quite far away" needs to be understood in the language describing micro world – it usually is a distance of a few nm), such a passing of energy has in approximation a Förster resonance character (Lakowicz, 1999; Kawski, 1992).

Similar mechanism of the transfer of the excitation energy takes place in diluted dye solutions (Lakowicz, 1999; Kawski, 1992). The energy returns among our antenna molecules are hardly probable - more probable is the roaming of the excitation between antennas with lower and lower excitation energy and after there trapping it in the reaction centre. The excitation energy in reaction centre is used for the charge separation and after further reactions is responsible among others for the difference of potentials between both surface of the membrane (Fig. 1). Some parts of the photosynthetic apparatus can be used in arrangements for conversion of light energy into electric energy. It is in simplification the description of the run of the photosynthetic process in such complex organisms as the higher plants.

Photosynthetic bacteria that appeared in the evolution period much earlier are simpler. There are many types of these bacteria in nature – such as purple, green, cyjanobacteria etc. Their antenna systems are very well adjusted to the range of light penetrating their existing environment. Some of them live in deep lakes and they take hydrogen not from the water decomposition but from sulphur compounds present in their environment:

$$nCO_2 + nH_2S \xrightarrow{h\nu} (CH_2O)_n + 2nS + nH_2O \qquad (2)$$

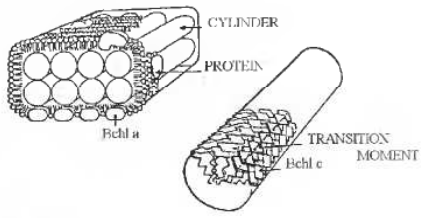

Fig. 2 Chlorosome structural model - green bacteria antenna complex.

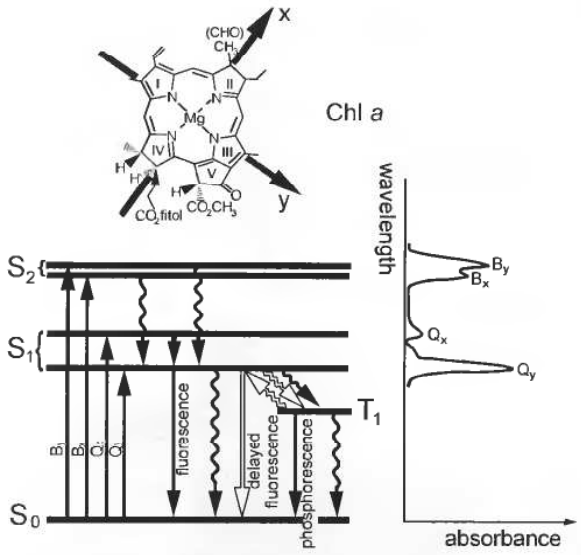

Fig. 3 Transition moments x and y locations in Chl a molecule skeleton; simplified Jablonski diagram of energy levels and main electronic transitions and absorbance spectrum of Chl a.

cules (Fig. 2). Dye molecules are placed so close to each other and exhibit such strong mutual interactions, that the process of excitation energy migration between them cannot be explained with the Förster resonance theory. Dexter theory can match better. According this theory the exciton migrates between molecules packed so closely that their electron clouds partly overlap one another. This theory described very well the excitation migration in molecular crystals. The antenna complexes of various organisms have different structure and differently packed pigments. Because of this Kenkre-Knox theory [6] tends to fit best the description of the migration of the excitation energy in biological systems. The theory assumes that there is an intermediate interaction between week (resonance — as in Förster's theory) and strong (Dexter's — exchange theory). In Kenkre and Knox's theory both of the above theories are included as limiting cases. How to recognise which reactions occur in a particular antenna complex? The answer gives the comparison between the theory and the experiments. If we know the distance between antennas (from structural experiments) and the probability of excitation transfer (from spectral analysis) then we can point out the dependence of probability of energy transfer from the distance between the donor and acceptor of energy (R). In Förster's resonance theory it is proportional to R^{-6}, in Dexter's theory to R^{-3} and in Kenkre-Knox theory index has an intermediate value dependent on the interaction strength among molecules.

All the above three theories have one characteristics in common : they take into account mutual arrangement of transition moments. The probability of energy transfer depend on the angle between the directions of the emission transition moment of

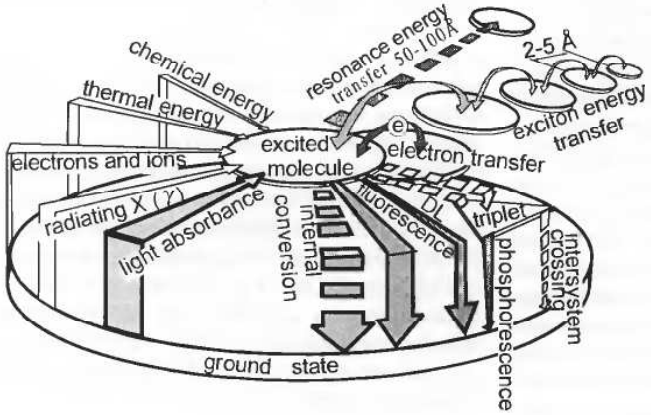

Fig. 4 The ways of molecule excitation and the paths of energy deactivation.

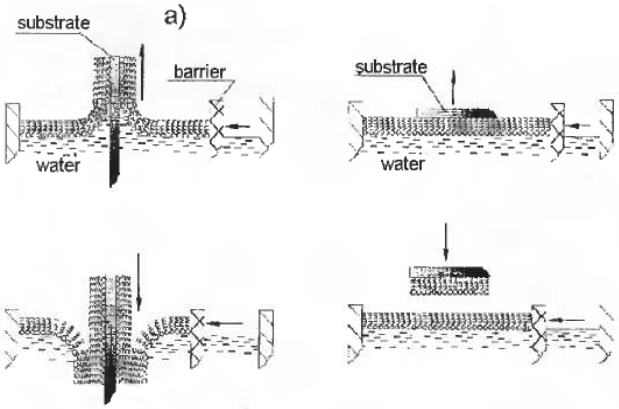

Fig. 5 Deposition of lipids monolayers by Langmuir-Blodgett method: (a) vertical „Y-type" method and (b) horizontal „X-type".

olecule being a donor energy and the absorption transition moment of the acceptor of excitation energy. It becomes highest when the transition moments are parallel and disappears when they are perpendicular. The absorption transition moment is connected with every one transition between electronic energy states. Consequently emission transition moment is related with the fluorescence emission. The positions of transition moments of the most important dye of plant -chlorophyll *a* located on the surface of porphyrin ring and the arrangement of energy levels in its spectrum of absorption and emission are shown in Fig. 3. Vibronic transition moments marked as x, cross I and II pyrrole rings, the ones marked as y – II and IV pyrrole. Fig. 3 shows also the position of O_x and Q_y bands in the range corresponding the absorption from state S_0->S_1 and B_x and B_y within the Soret band (S_0->S_2) (S — singlet state). Transition moments x and y are only approximately mutually perpendicular and the exact angle between them depends on groups attached to the ring as well as on the surroundings of chlorophyll molecule (Surma & Frąckowiak, 1970). The yield of the energy transfer between dyes depends strongly on mutual orientation of molecules being the donor and the acceptor of the energy. Due to the vast development of the molecular structure research given by the diffraction as well as the microscopic methods (i.e. Atomic Force Microscopy) (Binning & Rohrer, 1987) and also by ultra fast spectral methods (Holtzwarth, 1995; Zinth, Arlt, Hamm, Bibikowa, Dohse, Oesterhelt, Meyer & Scheer, 1995), one can presently compare experimental results of the migration of the excitation energy between antennas and the reaction centres with theoretical predictions. For chlorosomes (Fig. 2) Fetisova *et al.* (Novoderozhkin & Fetisova, 1995; Fetisova, Novoderzhkin, Taisova, Uzbekov, Freiberg, Tipmann & Belozersky, 1995) compared excitons properties achieved by "permanent hole burning" (meaning absorption spectrum changes of the sample illuminated by strong monochromatic radiation) with the assumption regarding the excitons properties taking into account different models of the interaction between bacteriochlorophyll *c* molecules. This method received the compatibility between the theory and the experience only for strongly interacting chains built out of dye molecules. A group of Japanese scientists reached the similar conclusion however with the assumption of different placement of these chains (Mimuro, Matsuura, Shimada, Nishimura, Yamazaki, Kolayashi, Wang, Nosava, 1995). Their investigation was based on the analysis of the fluorescence spectra. Our own research (Martyński, Frąckowiak, Miyake, Dudkowiak, Piechowiak, 1997), based on fluorescence microscopy as well as polarised light spectroscopy confirmed that the arrangement of Q_y transition moments is almost parallel to the axis of cylindrical rods (Fig. 2)

Fig. 3 presents Jablonski's diagram describing basic mechanisms of luminescence emission (fluorescence – F, delayed fluorescence – DF and phosphorescence – Ph). The molecule can be excited on a different way, but for the purpose of conversion of the light into electric energy it is necessary to absorb the electromagnetic radiation quanta. The system can return to the ground state also in a different way: by the luminescence emission (fluorescence, delayed luminescence), conversion of the excitation into heat (TD), excitation energy transfer to another molecule (ET), or through photochemical reaction (PCh) (Fig. 4). Antenna systems are characterised by high efficiency of the excitation transfer towards reaction centres and reaction centres are distinguished by high efficiency of charge separation reaction.

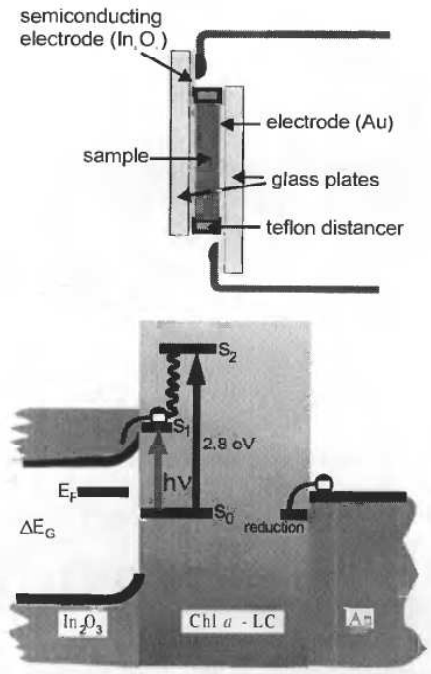

Fig. 6 Scheme of asymmetrical photoelectrochemical „sandwich" cell for the pigment photovoltaic properties measurements. Photopotential generation mechanism in asymmetrical cell with Chl *a* in liquid crystal (energy levels structure) (Dudkowiak *et al.*, 1996).

The sum of quantum efficiencies of all the paths of excitation energy deactivation equals:

$$\Phi_F + \Phi_{DL} + \Phi_{TD} + \Phi_{ET} + \Phi_{FCh} = 1 \qquad (3)$$

In our laboratory we can investigate all the above ways of deactivation of the absorbed energy. We are able to measure fluorescence, delayed fluorescence, phosphorescence, photothermal spectrum as well as the process of charge separation. Due to the complexity of the structure of the photosynthetic apparatus or even less complicated models of this apparatus, we use polarised light in investigation of all these spectra (Goc, Hara, Tateishi & Miyake, 1996; Frąckowiak, Zelent, Malak, Cegielski, Planner, Goc & Niedbalska, 1994; Enomoto, Takeda, Nakamura, Miyake, Ptak, Dudkowiak & Frąckowiak, 2000).

Now as we became familiar with photosynthesis secrets, we can pass to the constructions of the systems to the conversion of the light energy into the electric one using elements of photosynthetic organisms. What sort of conditions do these complexes have to fulfil in order to be economically justified? They need to show good enough conversion efficiency, have to fit within the sun spectrum reaching the Earth, be relatively cheap and be possibly stable in time. Also they should be environmentally friendly. The last condition is always fulfilled for these complexes, as the used elements of the organisms do not affect the environment similarly to the falling leaves. One has to work extremely hard in order to retain the semi biological complexes. The Japanese who co-operate with us have great achievements in this field. The efficiency of the conversion depends on mutual orientation of antennas molecules and reaction centres. In Goc works (Goc, Hara, Tateishi, Miyake, Planner & Frąckowiak, 1997; Miyake, Hara, Goc, Planner & Wróbel, 1997), carried out in cooperation with The National Institute of Advanced Interdisciplinary Research (NAIR) in Tskuba (Japan), antennas and the reaction centres were arranged in Lanmuir-Blodgett mono layers. Fig. 5 explains the rules of this method on an example of settling down of lipids. Mono layers were formed on the boundary of liquid "sub- phase" and the air (or another gas), and then deposited on quartz surface. We used native reaction centres of purple bacteria and chlorophyll *a* (plant pigment) as an antenna. Reaction centres and pigments were incorporated into liposomes - lipid vesicles popular also in cosmetics. Liposomes brought onto the sub-phase surface were getting deformed changing the round shape into the ellipsoidal one, then cracking forming two lipid layers with reaction centres and dyes. The series of the antenna-centre layers were placed on the semi-conductive electrode. The second electrode was made out of precious metal –gold or platinum. Both have to be in transparent to a large extent: the semi-conductive one in order to let the active light into electrochemical cell, metal one – to get rid of the light which has not been used to induce the antenna dye (Fig. 6). Excited antennas transfer their excitation energy to the reaction centres in which under the influence of oxidation they are able to transfer electrons to the conduction band of the semi-conductive electrode. The illuminated cell is thus the source of the electrochemical force. In order to reach the efficient system we have to join the whole series of these cells, as the output of the conversion in the single one is still low.

Do such systems have the future? Different sources of energy presently used have faults, such as: resources of the crude oil will soon run out and there are a lot of problems with the wastes of the nuclear reactors. Maybe we should, for example, put up small "roofs" out of photo-electrochemical cells over motorways and cover the roofs of houses with them, then we would obtain an ecological

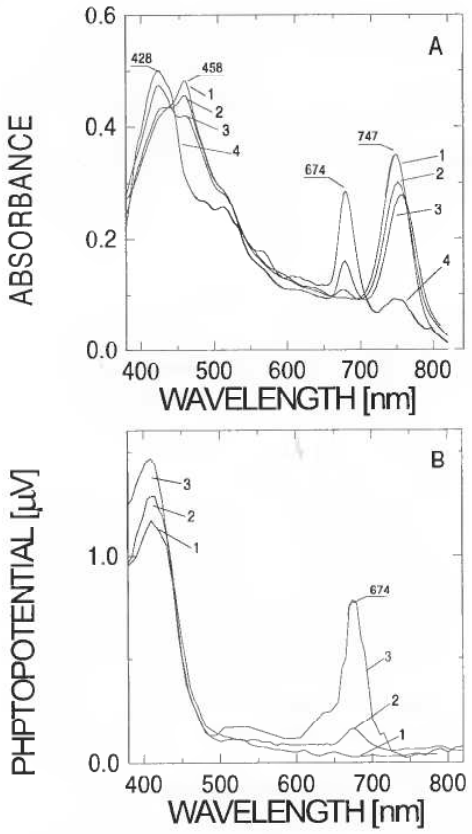

Fig. 7 Absorbance spectra (a) and photopotential action spectra (b) of green bacteria in electrochemical cell. The curves represent: (1) – native bacteria, (2)-(4) – bacteria with increasing intensity of oligomers desagregation (747 nm) (Ptak

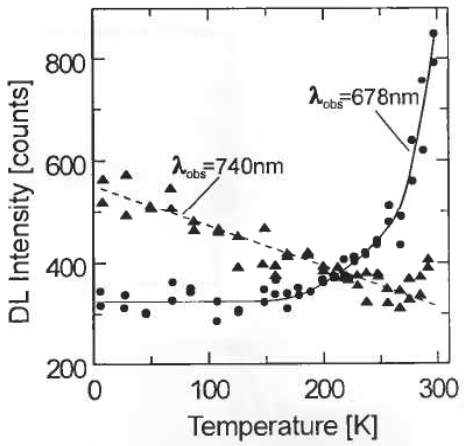

Fig. 8 Temperature dependence on delayed luminescence intensity of Chl *a* in PVA; (1) λ_{obs} = 678 nm, (2) λ_{obs} = 740 nm; c = 1,3 A 10^{-4} M, λ_{exc} = 418 nm, time window: 0,2 - 5,2 μs.

source of energy. It, of course, is the vision of the future but not that remote. The described model is one of very many tested by ourselves (Ptak, Chrzumnicka, Dudkowiak & Frąckowiak, 1996; Dudkowiak, Francke, Amesz, Planner, Hanyż & Frąckowiak, 1996a; Dudkowiak, Francke, Amesz, Planner & Frąckowiak, 1996b; Ptak, Dudkowiak & Frąckowiak, 1998). Practically each dye-protein complex set in the illuminated photo electrochemical cell can generate photopotential. The whole point is about constructing the most productive system. There are a lot of traps awaiting the constructors of such systems. For example – after placing whole green bacteria in the cell, photopotential action spectrum does not indicate the maximum connected with absorption of oligomers in the chromosome (747 nm) (Fig. 7). As the oligomer desagregates, the maximum connected with desaggregated dye (about 670 nm) increases in the absorption spectrum as well in the spectrum of photo potential action, whereas in the area of chlorosome oligomer absorption there is no recordable photopotential generation. It is connected with a very effective transfer of the excitation energy to the further parts of donor-acceptor chain of the conveying energy. The case is completely different if oligomers are placed directly in the electrochemical cell. Electrons are then transferred to the acceptor which is the semi-conducting electrode.

There is an next example of a dangerous interpretation presented in Fig. 8. The observed luminescence which is emitted by chlorophyll monomers is a delayed fluorescence (Fig. 3) emitted from singlet state after a thermal transition from T_1 $>S_1$ as it disappears with the decrease of the temperature. The emission of the luminescence of the aggregates has a completely different character, as its intensity increases together with the cooling of the sample. This emission comes from ions recombination leading to the excited state S_1 and delayed emission. Presented examples show that we need to establish very carefully the character of molecular processes undergoing in the examined model.

Another interesting way of technical use of photosynthetic process is hydrogen production through mutant strains of bacteria. The appropriately mutated bacteria, instead of producing hydro carbonates only for its own use, emit hydrogen very effectively. Hydrogen fuel is very interesting from the point of running of the vehicles. As we all know, burning hydrogen generates water so this process is environmentally friendly. In

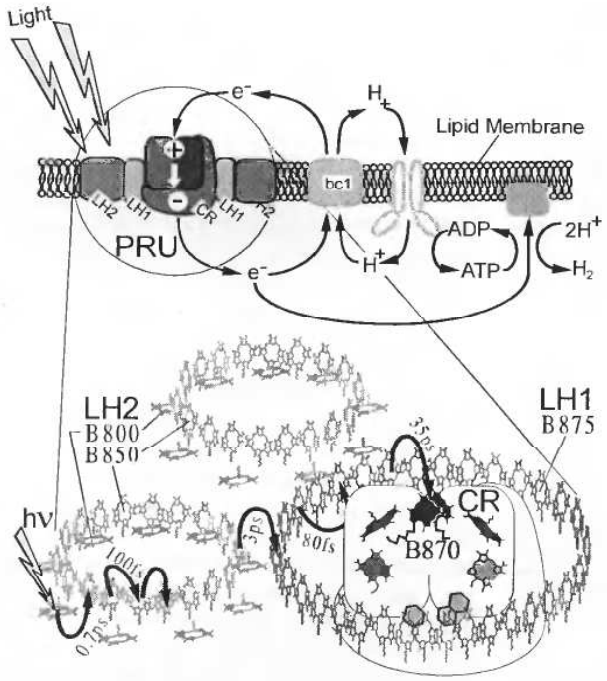

Fig. 9 Scheme of pigment-protein complexes orientation in lipid bilayer of purple photosynthetic bacteria. Arrangement of pigment molecules in photosynthetic unit (PRU) composed of reaction centre (CR), surrounded by antenna LH1 core complex and peripheral antenna complexes LH2.

collaboration with our Japanese colleagues we examined the efficiency of how the excitation energy in antennas reaches the reaction centres in mutated bacteria (Vasilieva, Miyake, Goc, Planner & Frąckowiak, 1999). Modified set of antenna complexes in mutants was different than in wild strain of the same bacteria. Contrary to the several antenna complexes LH1 of the wild strain of the bacteria which are usually placed in the nearest surroundings of the reaction centres (Fig. 9), mutants had fewer complexes LH1. Besides the transfer of the energy, for both types of bacteria strains we studied other ways of deactivation of the antenna excitation. It is very well known that antenna complexes are photo-chemically very stable, therefore their excitation energy which is not passed to the centres can be only emitted as luminescence (fluorescence or delayed luminescence) or also converted into heat. We therefore analysed photoacoustic spectra and, as a result of the division of the photoacoustic signal by the proper quantity of absorption, we obtained the efficiency of the thermal deactivation of individual types of antennas. We also measured fluorescence and delayed luminescence spectra at the excitation of individual dyes. After analysing these spectra we reached conclusions concerning the processes of the energy transfer between pigments of antenna and the reaction centres. It appeared that mutants, apart of having changes in the antenna complexes, cope very well with supplying of the excitation energy to the centres. Therefore such is their high efficiency of the hydrogen production (Vasilieva *et al.*, 1999).

Another way of applying the production of hydrogen was analysing how organisms do it and recreate similar molecular processes in an artificial system. In this case the physiological process was just a model of a technological process. H. Tien of USA (Goc & Tien, 1993) happened to choose this way. A partition, dividing two chambers – two watery areas, one of which was 3S electrolyte and the other – sea water, played the vital part in his device. Shotky's barrier acted as the model of membrane was built out of polycrystalline layer of cadmium selenide placed on a thin nickel foil (Fig. 10). The system was illuminated from CdSe side, which caused the shooting up of photo-

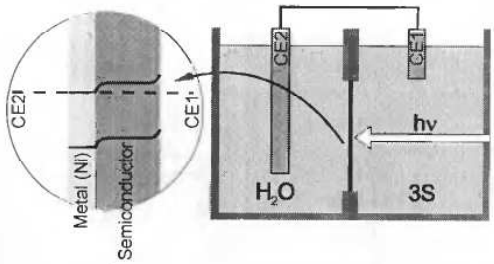

Fig. 10 Scheme of septum SC-SEP cell with Schotky barrier which play a rule of membrane dividing two aqueous solutions; structure of SC-SEP energy levels

at present – the production of hydrogen by the mutants of purple bacteria seems to be more promising (Vasilieva *et al.*, 1999). That is all as far as the application in the technology is concerned.

Now – the medicine. We shall only talk about so called photodynamic (PDT) therapy and diagnostics based on the experiments carried out in our laboratory. It is worth mentioning that this is only a small part of the application of biophysics in medicine. Presently there is no such physical method which is applied to the biological macro molecules experiments or the whole tissue, which would not have the use in medicine. For example – in The Institute of Nuclear Research in Cracow, under the guidance of professor A. Hrynkiewicz, a method of pointing out the elastic characteristics of the cells with the help of an atomic forces microscope, has been elaborated. This method, applied by Dr. M. Lekka lets us distinguish healthy cells and the cancer ones. The ones who are interested in the applications of biophysical methods in medicine, we would refer to books by Hrynkiewicz (1999, 2000).

In this article we shall confine ourselves, similarly as in the technical applications, just to the experiments which we carry out.

In photodynamic therapy one has to incorporate into tissue the photo sensitizer which after being highlighted, usually creates singlet oxygen $^1O_2$ strongly affecting tissues causing their degradation. Photo sensitizer useful in cancer diagnostics or therapy has to fulfil the following conditions:

(i) has to be absorbed selectively, meaning more by malignant tissues than by the healthy ones,

(ii) cannot be toxic for healthy cells and needs to be soon eliminated from them,

(iii) should efficiently produce singlet oxygen in cancer cells.

The first two conditions are important in diagnostics. The fluorescence of the sensitizer is very often used in diagnostics, as it is very important that it would stained the malignant cells. Fluorescent in a characteristic way areas of the infected cells are removed *via* an operation.

A tissue exhibit its own emission, which has to be distinguish from the sensitizer emission. The light is inserted into the internal organs through an optical wave-guide in order to excite the fluorescence. Similarly, it applies to the emitted light. A beam of light is submitted to a set of the complete internal reflections, moving this way along the optical wave-guide. Tissues apart from the fluorescence show a delayed luminescence. The generation of the fluorescence, delayed fluorescence and the phosphorescence is explained in Fig 3. All these types of the light emission have a very wide application in medicine. Illuminated healthy and malignant tissues show different light emission not only after applying the sensitizer on them, but they have their own different luminescence. Pictures observed under the fluorescence microscope supply us with more information about the distribution of different substances changing the fluorescence in the analysed tissue.

The reactions which take place in the tissue, which absorbed the molecule of the sensitizer P can be described in short in the following way:

$$P.(S_o) + h\nu \rightarrow P.(S_1) \rightarrow isc \rightarrow P.(T_1) \qquad (4)$$

Molecule of the sensitizer P in ground state is excited by light to singlet state and through the inter-system crossing (isc) transition can undergo to the triplet state.

It is known that the electron can stay fairly long on the level T_1, therefore there is a high probability to interact of the molecule P in triplet state with the

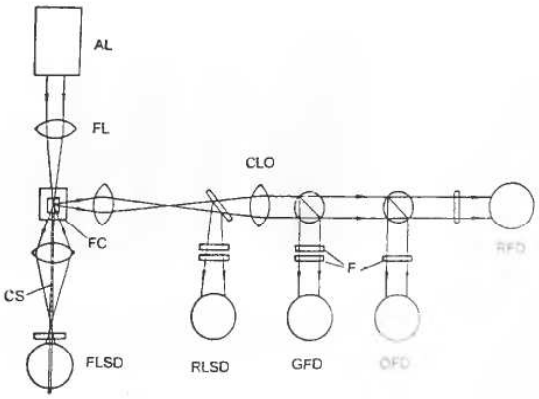

Fig. 11 Scheme of flow cytometry. AL – argon laser, FC – cell stream, FLSD i RLSD – detectors of light incident in front and perpendicular direction, GFD, OFD, RFD – fluorescence emission detectors (G – green, O – orange, R – red), F – filter (Wiktorowicz *et al.*, 1995).

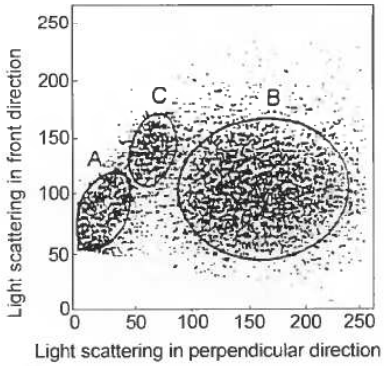

Fig. 12 Light scattering dependency on front and perpendicular direction for investigated sample (Frąckowiak *et al.*, 1998). A – lymphocytes area, B – granulocytes, C – monocytes.

oxygen molecule in triplet state according to the following reaction:

$$P.(T_1) + {}^3O_2 \rightarrow P.(S_o) + {}^1O_2 \qquad (5)$$

As the result we get very active molecules of singlet oxygen $^1O_2$, which destroy biological macromolecules, as well as the sensitizer ready for the use once again. In order for the photo-sensitive reaction to take place the light must get across the tissue to reach the sensitising dye, placed within this tissue. The dye has to absorb the light in the area hardly absorbed by the tissues. Such a "window" of absorption appears within the red and infrared (650 nm - 900 nm) range. Apart from that, the dye must be transferred to these tissues. Sometimes it is inserted with intravenous injection or by applying an ointment. In our experiments the cells are incubated in the dye solution outside the organism. Contrary to the expectations, the last method has a very wide clinical use. The bone marrow, collected form the leukaemia patient, can be incubated with the dye and if the dye is absorbed only by malignant leukocytes, then after being illuminated they get destroyed. After division of the remains of the broken leukocytes by centrifugation, the bone marrow may be inserted again into the spinal cord of the patient. Such a transplant is never rejected. Unfortunately, all dyes so far applied are introduced, to some extent also into healthy tissues and after the penetration of the light, destroy them as well. Therefore, we still search for the ideal photo sensitizer which would not only be inserted selectively into the infected tissues, but also destroy them. It is very interesting, that some pigments derivatives of photosynthetic organisms are good sensitizers. We carried out the research of the usefulness of various porphyrins and also of a large group of stilbazolium merocyanines (Ion, Planner, Wiktorowicz & Frąckowiak, 1999; Frąckowiak, Wiktorowicz, Cofta, Niedbalska & Latosińska, 1995; Wiktorowicz, Niedbalska, Planner, Frąckowiak, 1995). An examination of a large group of dyes allows us to come to more general conclusions, which show the way to further experiments. One can, for example, decide if neutral molecules are better or privileged are dyes with the charge, or as long chains attached to rings can help the penetration of molecules through molecular membranes. Somewhat there are some problems with the penetration of photo-sensitizers to the tissues, so sometimes one uses additional "vehicles" which help sensitizer molecules to get to the malignant cells. Liposomes are such "vehicles" in the middle of which a sensitizer can be present.

During our experiments we do not introduce of course new dyes into ill persons organisms but we bring them into so called resting or activated leukocytes (Frąckowiak, Planner, Ion & Wiktorowicz, 1998). Blood is taken from a healthy donor. Part of leukocytes are activated, it means incubated, not only with the dye but also with the substance which makes them resemble malignant cells. We analysed the structure of cell membranes that had been treated with different activators that we come across in literature. It turned out that their activity

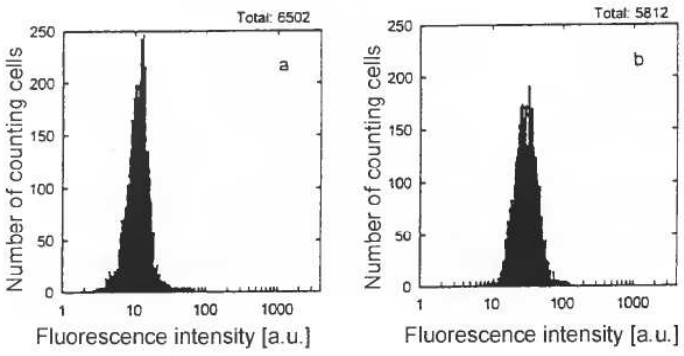

Fig. 13. Fluorescence intensity cytogramms of granulocytes incubated with merocyanines: mero – D_1 (a) and mero – A_1(b) (Frąckowiak *et al.*, 1998).

varies. Some very strongly interfere with the cell structure, in particular its membrane. Therefore, we cannot compare the results obtained for various activators: it is best to test them on blood samples of a truly ill donor. However, even here, we come across certain problems – the blood taken from different, even healthy donors, does not react in the same way with dyes and activators. How do we find a solution? One can do all sorts of experiments on blood obtained from different donors and only treat seriously such results which can be repeated in such conditions.

How do we examine penetration of the dye into cells and their possible lesion? The analysis of absorption, fluorescence, delayed luminescence and photo-thermal spectra come of an advantage. The latest show us which part of the light is exchanged into heat instead of producing singlet oxygen. Studying the spectra of the oriented cells using the polarised light provides us with information about cell structure. Flow cytometry plays also a very important role in experiments (Fig. 11). The stream of flowing cells is illuminated by almost parallel to it laser light beam. Depending on the size and properties of the cell surface, the number of quanta scattered along and across the beam changes. The picture of dependence of the number of quanta scattered in these directions supplies us with information about the properties of cells being examined. Fig. 12 shows areas of scattering for leukocytes, granulocytes and monocytes. The number of cells emitting fluorescence of a certain colour is also recorded (Fig. 13). The dependence of the number of counted cells on the intensity of emission in the given area of spectrum provides us with information about incorporation of dye into cells. The intensity of the fluorescence depends also on the efficiency of the emission of fluorescence dye in the cell. With the help of cytometer we directly get some information of possible cell lesion and about dye penetration into the sample. Photodynamic therapy presently develops very fast. We have previously (Frąckowiak *et al.*, 1998; Frąckowiak, Planner & Wiktorowicz, in press) described in more detail its application in different domains of medicine. Studying current programmes of international photo-biophysical conferences, we can assure ourselves that the subject together with the environment protection is highly in favour.

We hope that presented studies underline that Biophysics is useful not only in biological analysis but also has numerous practical aspects – both technical and medical.

Acknowledgments

D. Frąckowiak, J. Goc and A. Waszkowiak were supported by Polish KBN Grant 6 PO4A 010 17.

REFERENCES

Binning C. & Rohrer H. (1987). Skaningowa spektroskopia tunelowa od narodzin do wieku dojrzewania. *Postępy Fizyki* **38**, 493-502.

Dudkowiak A., Francke C., Amesz J., Planner A. & Frąckowiak D. (1996a). Spectral properties of BChl *c* in nematic liquid crystals. II: Aggregated form of dye. *Spectrochimica Acta Part A (Molecular Spectroscopy)* **52**, 1661-1669.

Dudkowiak A., Francke C., Amesz J., Planner A., Hanyż I. & Frąckowiak D. (1996b). Spectral properties of bacteriochlorophyll *c* in nematic liquid crystal. Part 1: Monomeric forms of dye. *Spectrochimica Acta Part A (Molecular Spectroscopy)* **52**, 251-264.

Enomoto H., Takeda S., Nakamura Ch., Miyake J., Ptak A., Dudkowiak A. & Frąckowiak D. (2000). Interaction of chlorophyll and polypeptide mixture with bacterial reaction centers. *Photosynthetica* **38**, 1-6.

Fetisova Z. S., Novoderzhkin V. I., Taisova A. S., Uzbekov R. E., Freiberg A. N., Tipmann K. E. & Belozersky A. N. (1995) Dynamics of a cylindrical exciton in chlorosomes of green bacteria. In:] *Photosynthesis: From Light to Biosphere.* (Ed.).*P. Mathis,* Kluver Acad. Publ. Vol. I, pp. 17-22.

Frąckowiak D., Dudkowiak A., Ptak A., Malak H., Gryczyński I. & Zelent B. (1998). Fluorescence liftimes of oriented green bacteria cells, cell fragments and and oriented bacteriochlorophyll *c* molecules. *J.Photochem. Photobiol., B: Biology* **44**, 231-239.

Frąckowiak D., Planner A., Ion R.–M., Wiktorowicz K., (1998). Incorporation of dye in restig and stimulated leukocytes. [In:] *Infrared dyes for high technology applications.* (Ed.) S. Daehne, Ute Resch – Genger and Otto S.Wolfbeis, Kluwer Academic Publishers, Dordrecht, Boston, London, pp.

Frąckowiak D., Planner A. & Wiktorowicz K. (in press). Near infrared applications in medicine. [In:] *Near infrared applications in biotechnology*, M. Dekker, Inc., New York, pp.

Frąckowiak D., Wiktorowicz K., Cófta J., Niedbalska M. & Latosińska M. (1995). Incorporation of stilbazolium merocyanines into resting and stimulated mononuclear leukocytes. *Acta Biochim. Polon.* **42**, 61-68.

Frąckowiak D., Zelent B., Malak H., Cegielski R., Planner A., Goc J. & Niedbalska M. (1994). Paths of deactivation of excitation of Chlorophyll a in various model systems, *SPIE* **2137**, 643-660.

Goc J. & Tien H. T. (1993). Electron and ion transport in SC-SEP cells with semiconductor thin film electrodes. *Int. J. Hydrogen Energy* **18**, 5-8.

Goc J., Hara M., Tateishi T. & Miyake J. (1996). Reconstructed light-harvesting system for photosynthetic

reaction centres. *J. Photochem. Photobiol. A: Chem.* **93**, 137-144.

Goc J., Hara M., Tateishi T., Miyake J., Planner A. & Frąckowiak D. (1997). Spectral properties of the photosynthetic reaction units reconstituted from bacterial reaction centers and antenna pigments located in liposomes suspended in buffer or ordered in Langmuir – Blodgett films. *J. Photochem. and Photobiol. A: Chem.* **104**, 123-131.

Holtzwarth A. (1995). Ultrafast spectroscopy of the light-harvesting complex and isolated reaction center from photosystem II, [In:] *Photosynthesis: From Light to Biosphere.* (Ed.) P. Mathis, Kluver Acad. Publ., Vol. I, pp. 35-40.

Hrynkiewicz A. (1999). *Fizyczne metody badań w biologii, medycynie i ochronie środowiska*. PWN, Warszawa (1999).

Hrynkiewicz A. (2000) *Fizyczne Metody Diagnostyki Medycznej i Terapii*, PWN, Warszawa.

Ion R. M., Planner A., Wiktorowicz K. & Frąckowiak D. (1999). The incorporation of various porphyrins into human blood cells measured via flow cytometry, absorption and emission spectroscopy, *Acta Biochim. Polon.* **45**, 833-845.

Kawski A. (1992). *Fotoluminescencja roztworów*, PWN, Warszawa.

Kenkere K. M., Knox R. S. (1974). Theory of Fast and Slow Excitation Transfer rates. *Phys. Rev. Lett.* **33**, 803-818.

Lakowicz J. R. (1999). *Principles of Fluorescence Spectroscopy*. [Ed.] Kluwer Acad. Publ., New York, Boston, Dodrecht, London, Moskow.

Martyński T., Frąckowiak D., Miyake J., Dudkowiak A. & Piechowiak A. (1997). The orientation of bacteriochlorophyll c in green bacteria cells and cell fragments. *J. Photochem. Photobiol. B* **42**, 57-66.

Mimuro M., Matsuura K., Shimada K., Nishimura Y., Yamazaki I., Kolayashi M., Wang Z. Y. & Nosava T. (1995). Molecular network and funneling process of energy transfer in green photosynthetic bacteria. [In:] *Photosynthesis*In:] *Photosynthesis: From Light to Biosphere.* (Ed.).*P. Mathis,* Kluver Acad. Publ. Vol. I, pp. 41-46.

Miyake J., Hara M., Goc J., Planner A. & Wróbel D. (1997). Deactivation of excitation energy in bacterial photosynthetic reaction centers in Langmuir – Blodgett films. *Spectrochimica Acta* Part A **53**, 1485-1493.

Novoderozhkin V. H. & Fetisova Z. G. (1995). Spectral hole burning as a test for exciton models of chlorosomal antenna. In:] *Photosynthesis: From Light to Biosphere.* (Ed.).*P. Mathis,* Kluver Acad. Publ. Vol. I, pp. 99-102.

Planner A., Susła B., Nowicki M., Klaczyńska K. & Frąckowiak D. (1999). Paths of bacteriochlorophyll *c* deexcitation in green photosynthetic bacteria and in model system. *J. Fluorescence* **9**, 139-143.

Ptak A., Chrzumnicka E., Dudkowiak A. & Frąckowiak D. (1996). Electrochemical cell with bacteriochlorophyll *c* and chlorophylls *a* and *b* in nematic liquid crystal. *J. Photochem. Photobiol. A: Chem.* **98**, 159-163.

Ptak A., Dudkowiak A. & Frąckowiak D. (1998). Photoelectrochemical properties of green bacteria cells and cell fragments located in electrochemical cell. *J. Photochem. Photobiol. A: Chem.* **115**, 63-83.

Surma S. & Frąckowiak D. (1970). Optical Properties of Chlorophyllide in Anisotropic Medium, *Photosynthetica* **4**, 202-213.

Vasilieva L., Miyake J., Goc J., Planner A. & Frąckowiak D. (1999). The paths of excitation energy deactivation in LH1 deficent mutant and wild strains of Rhodopseudemonas spheroides. *J. Fluorescence* **9**, 347-355.

Wiktorowicz K., Niedbalska M., Planner A. & Frackowiak D. (1995). Incorporation of stilbazolium merocyaninines into human leukocytes measured by flow cytometry. *Acta Biochim. Polon.* **42**, 333-338.

Wróblewski A. K., *Uczeni w anegdocie*, Prószyński i S-ka, Warszawa 1999.

Zinth W., Arlt T., Hamm P., Bibikova M., Dohse B., Oesterhelt D., Meyer M., Scheer H. (1995) Ultrafast infrared and visible spectroscopy of bacterial reaction centers. [In:] *Photosynthesis: From Light to Biosphere.* (Ed.).*P. Mathis,* Kluver Acad. Publ. Vol. I, pp. 389-394, (1995)