EESTI NSV TEADUSTE AKADEEMIA TOIMETISED.
FÜÜSIKA. MATEMAATIKA
ИЗВЕСТИЯ АКАДЕМИИ НАУК ЭСТОНСКОЙ ССР. ФИЗИКА. МАТЕМАТИКА
PROCEEDINGS OF THE ACADEMY OF SCIENCES OF THE ESTONIAN SSR.
PHYSICS. MATHEMATICS
1984, 33, 2

УДК 535.373.3

Valentina GODIK, A. FREIBERG, K. TIMPMANN

PICOSECOND SPECTROCHRONOGRAPHY STUDY OF LIGHT ENERGY UTILIZATION BY PHOTOSYNTHETIC BACTERIA

(Presented by K. K. Rebane)

Bacteriochlorophyll (BChl) fluorescence lifetimes (τ) of purple bacteria were measured at low exciting pulse energy with the use of a picosecond fluorescence spectrochronograph of high sensitivity and high time resolution. The whole light intensity dependence of τ was followed for *Rhodospirillum rubrum* chromatophores from low (nearly all the reaction centres (RCs) open) to saturating light; the time interval from light absorption to excitation trapping by a RC was measured to be 50 ± 10 ps. Selective excitation of *R. rubrum* chromatophores by light, absorbed by RCs ($\lambda\approx800$ nm) or by antenna ($\lambda=370-380$ nm) BChl, made it possible to show that excitation energy transfer in bacterial photosynthesis is a trap-limited, rather than a diffusion-limited process. The time of the primary charge separation *in situ* was estimated to be in the range of 10—20 ps.

1. Introduction

Light quanta absorbed by photosynthetic organisms create singlet excited states of chlorophylls or accessory pigments. Due to intermolecular coupling between pigment molecules in the photosynthetic units (PSU), these excited states are not localized but are rather hopping between the molecules of the light-harvesting antenna until captured by RC. At the RCs, excitation energy is transformed step-by-step into electrochemical energy of separated charges. It is believed that excitation energy transfer *in vivo* occurs via the mechanism of resonant energy transfer (for review, see [1, 2]). This process plays an important role in photosynthesis, determining, in total, the quantum yield of sun light utilization. Despite numerous theoretical and experimental studies of energy transfer and trapping, the mechanisms of these two key photosynthetic processes are not completely understood.

A powerful experimental approach to the above problem is time-resolved fluorescence spectroscopy (see reviews [1–3]). The first τ measurements of the photosynthetic objects were performed with the use of the phase-shift method (described, for example, in [2]) which is quite an adequate mode for studying exponentially decaying emissions. For analysis of multicomponental emissions, some additional suppositions are needed in order to obtain the kinetics of individual components. Thus, according to [4, 5], τ values about 1 ns, usually measured for purple bacteria by the phase-shift method, do not pertain to the bulk BChl but rather to some minor long-lived component. The bulk BChl τ for RCs open was estimated to be about 50 ps. Similar data were recently obtained in [6]. Another, more direct, way of τ measurements is laser spectro-

fluorimetry with picosecond resolution, which allows one to follow directly the whole fluorescence decay curve [7, 8]. However, the first investigations performed by this method showed that the results obtained are not easily interpreted as the ultrashort light pulses used were rather intense. Thus it was hardly possible to exclude a possibility of finding simultaneously two or more excitations in PSU, especially as a number of PSUs may be interconnected at the level of excitation energy transfer. On natural conditions such situation is never realized. To overcome this difficulty a novel highly sensitive picosecond resolution spectrochronography method [9] as well as a single-photon timing with ~ 0.1 ns resolution [10] were recently employed to follow the fluorescence decay kinetics of plant objects. Low intensity level was reliably achieved and important data obtained. However, since the objects employed contained two photosystems, the observed decay kinetics were greatly complicated by excitation energy and electron transfer interactions between them.

This work describes the results of the study of the primary steps of bacterial photosynthesis at ambient temperature with the use of a fluorescent picosecond spectrochronograph which allows measurements of fluorescence decay curves over a wide spectral range with high time and spectral resolution under conditions of very low excitation pulse energy. As a main object for the study of excitation transfer and trapping native chromatophores from purple bacterium *R. rubrum* were selected, since this bacterium contains the simplest light-harvesting antenna with a single absorption band B_{880}. Parallel with the picosecond lifetime measurements, the dependence of fluorescence yield φ and the portion of photooxidized reaction centres (P^+/P_0) on the exciting light intensity were measured. Some preliminary results obtained on cells of sulfur purple bacterium *Chromatium minutissimum* are also given.

2. Experimental

2.1. Objects. Cells of *R. rubrum* (wild type No. 1 MGU) were grown and chromatophores were isolated as described elsewhere [11]. Chromatophore suspensions with an optical density of A_{880} nm/ 1 cm$\approx$ 200 were stored at 0° C under anaerobic conditions and diluted by the buffer to a final BChl concentration of $3-6\cdot10^{16}$ molecules/ml. The measuring solution contained 220 mM sucrose, 50 mM Tris-HCl buffer (pH 7.5) and 2mM $MgSO_4$. Cells of *Chr. minutissimum* were grown in the modified Larsen's medium and used 3—5 days after inoculation from a previous culture.

2.2. Methods. Picosecond spectrochronograph with 5 ps time and less than 1 nm spectral resolution in 350—1050 nm range [9, 12, 13] is based on a combination of a «Spectra-Physics» mode locked cw oxazine 1 or oxazine 750 dye laser (tuning range 685—820 nm, 345—410 nm with frequency doubling, pulse duration 3 ps, average power up to 200 mW) synchronously pumped with 82 MHz by a krypton-ion laser, a double-grating spectrometer and a streak camera (Fig. 1). A subtractive dispersion mount of spectrometer is used to narrow its pulse response with preserved luminosity up to the transform-limited value [13]. For data recording and processing, a EC 1010-computer-controlled B&M Spektronik OSA500 optical multichannel analyzer with SIT vidicon is used. Instead of commonly used pulsed deflection of streak camera, a continuous streaking in synchronism with the dye laser operation [14] is employed. As a result, a high quality of a set of important parameters is achieved in our set-up (see Fig. 1). For measurements of τ light-intensity dependence, the analyzing monochromator was changed for narrow-band interference and glass filters; in some cases a thin plate of GaAs

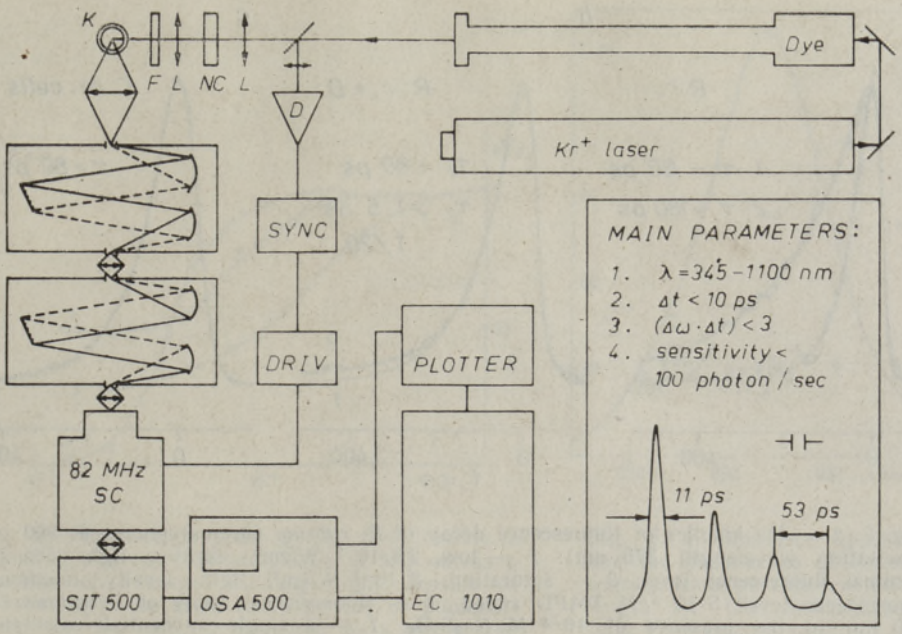

Fig. 1. Picosecond spectrochronograph for studying time resolved emission spectra. L — lens; NC — nonlinear crystal for second harmonic generation; F — filters; K — sample; SC — synchroscan streak camera; D — photodiode; SYNC — synchronization unit; DRIV — streak camera driver.

was used. Fluorescence was viewed at an angle of ~90° to the exciting light direction in a reflection mode. The average exciting light density was changed with the help of gray filters and checked by a radiometer NRC Model 880, or a calibrated photoelement. The same detector was used to measure continuous light intensity in measurements of φ and P^+/P_0. The latter parameter was measured as a normalized photobleaching at 865 nm with a double-beam differential spectrophotometer. The φ measurements were made with an Aminco-Bowman spectrofluorimeter [5]. All fluorescence decay curves were treated as a sum of two exponentials taking into account the apparatus response.

3. Results

3.1. Light-intensity dependence of *R. rubrum* fluorescence lifetime. Fluorescence decay curves of *R. rubrum* chromatophores excited by 375 nm light pulses of low ($6 \cdot 10^5$ quanta/cm²/pulse) and saturating ($5 \cdot 10^7$) intensities are shown in Fig. 2, *a*. Both experimental curves are well approximated by a single exponential component (the same is true of intermediate intensities), and 1/*e* time, τ, is equal to $\tau^{min} = 50$ ps for low, and $\tau^{max} = 190$ ps for saturating light. This agrees well with earlier data [1, 5]. Repeated measurements with a given sample gave the values that agree within 10% (including day-to-day variations), but sample-to-sample variations were up to 40% (see Fig. 3). The measured lifetime in one of the samples is presented in Fig. 4 (curve *2*) as a function of average exciting light density. The results of parallel measurements of φ and P^+/P_0 dependences on continuous light intensity for the same sample are also plotted in Fig. 4. It can be seen that as the RCs turned into the closed state, τ and φ increased nearly parallel. Maximal increase

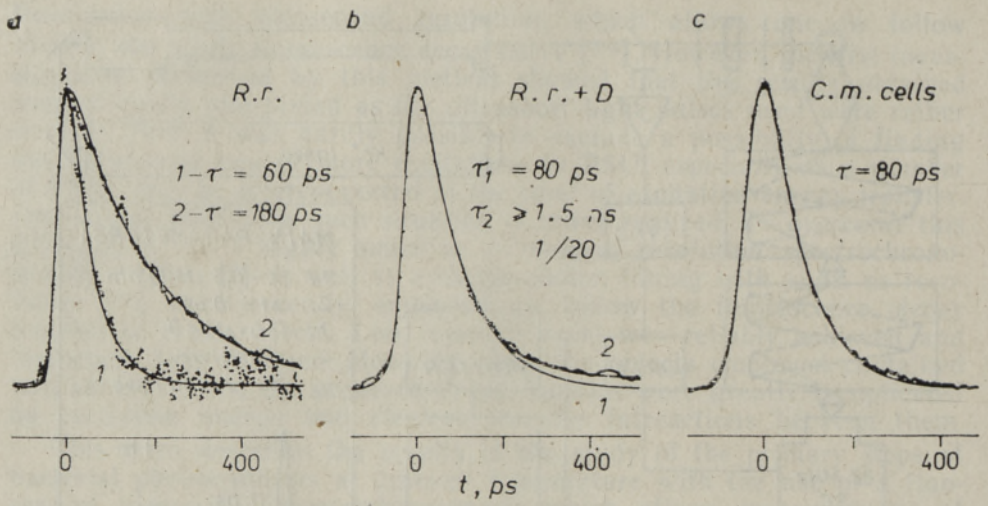

Fig. 2. *a* — the kinetics of fluorescence decay of *R. rubrum* chromatophores at 900 nm (excitation wavelength 375 nm): *1* — low, $2.5 \cdot 10^{-5}$ W/cm², exciting light density, minimal fluorescence level; *2* — saturating, $2 \cdot 10^{-3}$ W/cm², light density, maximum fluorescence level; $5 \cdot 10^{-5}$ M TMPD added. *b* — fluorescence decay of *R. rubrum* at 900 nm in the presence of 10^{-3} M $Na_2S_2O_4$: *1* — a single-exponential calculated response with $\tau = 80$ ps; *2* — two-exponential approach with amplitude ratio $A_2/A_1 = 1/20$; exciting light density $5 \cdot 10^{-3}$ W/cm² at $\lambda = 800$ nm. *c* — fluorescence decay kinetics of *Chr. minutissimum* cells at 920 nm; excitation wavelength 750 nm, light density $3 \cdot 10^{-3}$ W/cm². Dotted curves are experimental, continuous ones are the calculated fluorescence responses.

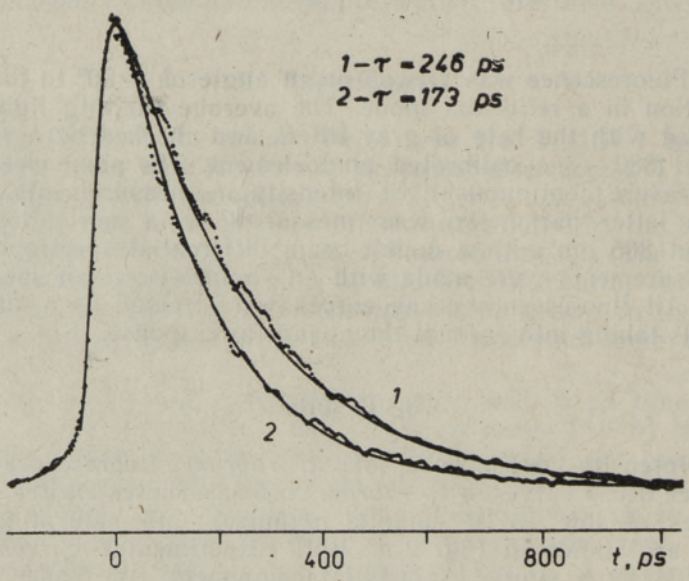

Fig. 3. The kinetics of fluorescence decay of two different preparations of *R. rubrum* chromatophores under excitation with 800 nm exciting light of saturating density, $2 \cdot 10^{-3}$ W/cm². Continuous and dotted curves are the same as in Fig. 2.

in τ observed by us was 3—4 times. Above the saturating light intensity level (intensities up to 3 W/cm² were employed for $\lambda = 750$ nm), τ^{max} did not change. This means that neither singlet-singlet nor singlet-triplet annihilation took place in our experiments.

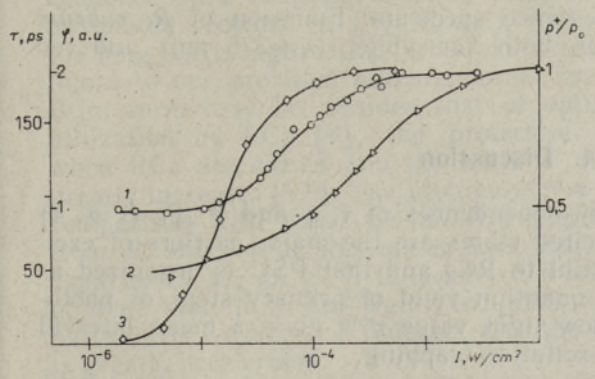

Fig. 4. Fluorescence yield φ (*1*) and lifetime τ (*2*) of *R. rubrum* chromatophores at 902 nm as a function of average exciting light density at 375 nm; (*3*) the same for a portion of photooxidized reaction centres, measured as a normalized absorption change P^+/P_0 at 600 nm. Actinic light with $\lambda \geqslant 700$ nm was used in the latter case.

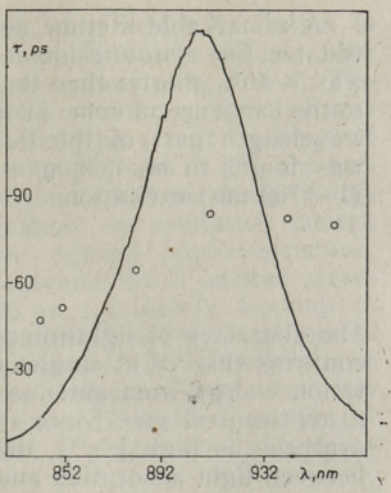

Fig. 5. Fluorescence spectrum and the dependence of fluorescence lifetime on emission wavelength for *Chr. minutissimum* cells. Fluorescence spectrum was measured in 1 cm — cell, optical density at $\lambda = 365$ nm was 0.2. Lifetimes were measured with 755 nm exciting light of density, 1 W/cm².

3.2. Long-lived nanosecond components in the emission of *R. rubrum*. Under certain conditions, together with the picosecond component of the emission described above, a nanosecond one was also observed. Two-exponential decays were detected for *R. rubrum* in the following cases:

(1) Chromatophore suspensions in the presence of 10^{-3} M $Na_2S_2O_4$ (Fig. 2, *b*);

(2) Chromatophore suspensions after addition of reducing agents, such as sodium ascorbate, $2 \cdot 10^{-3}$ M, or mercaptonol, 10^{-3} M and N, N, N′,N′-tetramethyl-p-phenylendiamine (TMPD). TMPD concentration was varied in the range of $5 \cdot 10^{-6} - 5 \cdot 10^{-4}$ M in order to obtain a necessary shift of the light intensity curves to higher intensities. These TMPD additions have no noticeable effect on the measured value of τ or the character of fluorescence decay when reducing agents were absent in the measuring solution;

(3) Intact cells of *R. rubrum* without any additions.

In all cases the lifetime of the second component was about 2 ns and its relative amplitude was less than ten per cent. The lifetime of the shorter component does not change substantially in any samples.

3.3. Fluorescence lifetime of purple bacteria as a function of excitation and emission wavelength. Such measurements are especially informative when a possibility of selective excitation of an appreciable part of RCs can be realized. Three excitation wavelengths were used in our experiments: (1) $\lambda = 375 - 378$ nm, which excites antenna BChl (RC absorption is negligible); (2) $\lambda = 798 - 800$ nm, at this wavelenght RCs in *R. rubrum* absorb more than half of the exciting light; (3) $\lambda = 760$ nm, which excites primarily RC bacteriopheophytine. Fluorescence decay curves for all these wavelengths were superimposible both for low and saturating light intensities. A one-exponential fluorescence decay of *Chr. minutissimum* cells (Fig. 2, *c*) was also independent of the excitation wavelengths.

A remarkable lifetime dependence on emission wavelength was observed for *Chr. minutissimum* (Fig. 5): τ for the short wavelength range was $\sim 40\%$ shorter than that for the long wavelength range, which points to the existence of some short-lived ($\tau \sim 10$ ps) component(s) at the short-wavelength part of the fluorescence spectrum. Emission of *R. rubrum* was found to be homogeneous both for violet ($\lambda = 375$ nm) and red ($\lambda = 760$ nm) excitations.

4. Discussion

The character of light-intensity dependences of τ, φ and P^+/P_0 (Fig. 4) confirms that BChl singlet excited states are the major carriers of excitation energy from antenna BChl to RCs and that PSU is organized as a multicentral one. Since the quantum yield of primary steps of photosynthesis is high [15, 16], the low light value τ^{min} gives a mean interval between light absorption and excitation trapping.

Two extreme cases are distinguished depending on what is the bottleneck of the whole process: diffusion of excitations from antenna to RC (efficiency of excitation trapping by RC from the first visit is high) or the rate of the primary charge separation process (excitation visits RC many times before trapping). There has been no unequivocal evidence of whether diffusion or trap-limited migration is realized in purple bacteria. Fluorescence lifetime measurements under conditions of selective excitation of RC or of antenna BChl may solve the problem, provided exciting light intensity is sufficiently low [17].

In our experiments with *R. rubrum* chromatophores were selectively excited by light with $\lambda = 798$, 758 and 375 nm. The fact that fluorescence decay curves for these wavelengths were superimposible shows that, no matter what is primarily excited, RC or antenna BChl, the decay of excitations proceeds in the same way. This means that equilibrium distribution of excitations between RC and antenna BChl takes place before excitation is irreversibly trapped. In the alternative case τ^{min} should be shorter when part of excitation quanta is absorbed directly by RCs. Thus, in *R. rubrum* a purely trap-limited case of excitation energy migration is realized. In this case, τ^{min} is simply related to τ_e, *in situ* charge separation lifetime, namely $\tau_e = \tau^{min}/N$ [18], where N is the number of antenna BChl molecules per RC. Since in our *R. rubrum* chromatophore preparations N was equal to 35—40, τ_e should be equal to 1—2 ps. In case of trap-limited migration, the pairwise jump time τ_j between adjacent BChl molecules should be approximately an order of magnitude less than τ_e, i.e. of the order of 10^{-13} s.

This time is rather short in the view of the known data on PSU organization in the photosynthetic membranes. Namely, it is believed that BChl molecules B_{880} of *R. rubrum* as well as B_{860} of *Rhodopseudomonas sphaeroides* R-26 are arranged as dimers bound to hydrophobic proteins with a molecular weight between 10,000 and 20,000 [19]. Taking into account the size of these pigment-protein globules, an average intermolecular distance between the chromophores bound to adjacent globules, should be about 20 Å or larger. According to Förster's formula: $\tau_j = \tau_0 (R/R_0)^6 \cdot q^{-1}$ ($\tau_0 \simeq 18$ ns is the intrinsic BChl lifetime, R_0 Förster's radius, equal for BChl to 80—90 Å [8], q, the number of nearest neighbour acceptors), τ_j for such intermolecular distances should be not shorter than several picosecond. We believe that the apparent contradiction we come to is due to the fact that fast excitonic exchange between RC and antenna BChl holds not for a whole PSU, but between RC and a small limited number of BChl molecules adjacent to it. These molecules form a

«proximal», tightly coupled with RC antenna, over which excitation is delocalized during the charge separation process. If this is the case, the above estimations would give somewhat larger values of τ_e and τ_j. The existence of a coupling antenna complex with similar properties was postulated recently in [20]. It may well be that the recent findings of the long wavelength BChl forms of *R. rubrum* in [21] and in [22] are pertinent to our proximal antenna form. The physiological meaning of this BChl form may be, besides that of optimization of excitation energy utilization by RCs [23], the protective role against photodestruction, when RCs are closed and the lifetime of antenna BChl excited states greatly increase [5, 24]. The lifetime $\tau^{max} \approx 200$ ps is, evidently, too short in comparison with those for RC-free pigment-protein antenna complexes from *R. rubrum* or RC-less mutant *Rps. sphaeroides* PM-8 where τ is equal to 1—1.5 ns [5, 8]. It is known from the studies on photochemistry of dyes that, if in the tightly coupled molecular complex one of the partners is oxidized or reduced, and the other is photoexcited, its decay is greatly accelerated.

In conclusion, our data are summarized as the following scheme of structure-function organization of excitation energy transfer and trapping in *R. rubrum*. Several (4—10) RCs are interconnected with each other via their proximal antenna BChl, which form, together with adjacent B_{880} molecules, photosynthetic domain. Excitation from B_{880} quickly reach the proximal antenna and then RC, but since the efficiency of trapping from the first visit is low and interaction inside RC-proximal antenna complex is high, excitation remains in this complex during 50—70 ps until trapped by a RC. As RCs become closed by photooxidation, excitation lifetime progressively increases, since there is now a larger number of BChl molecules of the proximal antenna per open RC. The data supporting the above scheme, which were recently obtained in [21, 22] show that in *R. rubrum* there is a minor (3—5 molecules per RC) form of antenna BChl some 10—12 BChl molecules. Taking into account these data, τ_e may be estimated as 10—20 ps, which is several times larger than τ_e values measured by absorption spectroscopy for isolated RCs [25].

In the light of the results obtained in this work it becomes clear why inconsistencies in τ and φ dependences on exciting light intensity are encountered when these are compared for low-intensity CW picosecond pulse and relatively high-intensity single-pulse picosecond excitations. Firstly, the results at equal pulse (not average) energy should be compared. Secondly, in the case of single-pulse excitation quanta come to a photosynthetic domain almost simultaneously, and when excitation light density is low enough (less than one quantum per photosynthetic domain), all quanta are localized in the RC-proximal antenna complex until excitation trapping occurs. At higher pulse energies, besides trapping, efficient annihilation due to rather strong excitonic coupling between BChl molecules of the proximal antenna takes place. As a result, φ will be constant when the number of quanta/domain $\leqslant 1$, and then decrease [8]. Nearly the same is true with lifetime dependence on excitation intensity. In the case of recurrence excitation with pulse-sequence period less than biochemical recovery time of the PSU as well as of continuous excitation the effect of excitation accumulation will work. As a result, already at relatively low pulse intensities the rise of quantum yield and lifetime is observed which saturates at higher intensities.

The nature of nanosecond components in the emission of purple bacteria as well as the *Chr. minutissimum* emission heterogeneity, will be discussed elsewhere.

5. Conclusions

1. Employment of a new experimental technique, picosecond spectrochronography, made it possible to measure, for the first time, the fluorescence kinetics of purple bacteria with picosecond resolution and average excitation light density close to natural.

2. The whole τ light-intensity curve was followed from low (RCs open) to saturating light, and the time from light absorption to excitation trapping was measured to be 50 ± 10 ps. It is shown that τ and φ increase in parallel as RCs turn into the closed state.

3. Selective excitation with light, absorbed by RCs, or by those absorbed by antenna BChl, shows that excitation energy transfer in *R. rubrum* is a trap-limited rather than a diffusion-limited process.

4. The time of primary charge separation *in situ* was determined to be in the range of 10—20 ps, and the pairwise jump time of excitation energy transfer was in the range of picoseconds.

5. To reconcile the data obtained with the known data on the structure of the photosynthetic apparatus, it is necessary to propose the existence of a proximal, tightly coupled with RCs antenna complex, distinct from the major B_{880} one, which includes several BChl molecules.

6. The emission of *R. rubrum* was found to be spectrally homogeneous, while those of *Chr. minutissimum* was inhomogeneous.

Acknowledgements. The authors are indebted to Profs K. K. Rebane and A. Yu. Borisov for valuable discussions and support, to Dr. S. G. Kharchenko for preparation of chromatophores.

REFERENCES

1. *Borisov, A. Yu., Godik, V. I.* Biochim. Biophys. Acta, **301**, 227—248 (1973).
2. *Knox, R. S.* In: Bioenergetics of Photosynthesis (Ed. Govindjee), Chp. 4, New York, Academic Press, (1975).
3. *Rubin, A. B.* Photochem. Photobiol., **28**, 1021—1040 (1978).
4. *Borisov, A. Yu., Godik, V. I.* Biochim. Biophys. Acta, 223, 441—443 (1970).
5. *Godik, V. I., Borisov, A. Yu.* FEBS Lett., **82**, 355—358 (1977).
6. *Sebban, P., Moya, I.* Biochim. Biophys. Acta, **722**, 436—442 (1983).
7. *Paschenko, V. Z., Protasov, S. P., Rubin, A. B., Timofeev, K. N., Zamazova, L. M., Rubin, L. B.* Biochim. Biophys. Acta, **408**, 143—153 (1975).
8. *Campillo, A. J., Hyer, R. C., Monger, T. G., Parson, W. W., Shapiro, S. L.* Proc. Nat. Acad. Sci. USA, **74**, 1997—2001 (1977).
9. *Freiberg, A. M., Timpmann, K. E., Tamkivi, R. P., Avarmaa, R. A.* ENSV TA Toim. Füüs. Matem., **31**, 200—207 (1982).
10. *Haehnel, W., Nairn, J. A., Reisberg, P., Sauer, K.* Biochim. Biophys. Acta, **680**, 161—173 (1982).
11. *Nazarenko, A. V., Samuilov, V. D., Skulachev, V. P.* Biokhimiya **36**, 780—782 (1971).
12. *Freiberg, A., Saari, P.* IEEE J. Quant. Electron., **QE-19**, 622—630 (1983).
13. *Saari, P., Aaviksoo, J., Freiberg, A., Timpmann, K.* Opt. Commun., **39**, 94—98 (1981).
14. *Freiberg, A., Raidaru, A., Anijalg, A., Timpmann, K., Kukk, P., Saari, P.* ENSV TA Toim. Füüs. Matem., **29**, 187—194 (1980).
15. *Loach, P. A., Secura, D. L.* Photochem. Photobiol., **1**, 2642—2649 (1968).
16. *Barsky, E. L., Borisov, A. Yu.* J. Bioenerg., **2**, 275—280 (1971).
17. *Pearlstein, R. M.* Photochem. Photobiol., **35**, 835—844 (1982).
18. *Montroll, E. W.* Proc. Symp. Appl. Math. **16**, 193—220 (1964).
19. *Sauer, K., Austin, L.* Biochemistry, **17**, 2011—2019 (1978).
20. *van Grondelle, R., Duysens, L. N. M.* Plant Physiol, **65**, 751—754 (1980).
21. *Borisov, A. Yu., Gadonas, R. A., Danielius, R. V., Piskarskas, A. S., Razjivin, A. P.* FEBS Lett., **138**, 25—28 (1982).
22. *Gómez, I., Sieiro, C., Ramirez, J. M., Gomez-Amores, S., del-Campo, F. F.* FEBS Lett., **144**, 117—120 (1982).

23. *Fetisova, Z. G., Fok, M. V., Borisov, A. Yu.* Molek. Biol., **17**, 437—445 (1983).
24. *Heathcote, P., Clayton, R. K.* Biochim. Biophys. Acta, **459**, 506—515 (1977).
25. *Holten, D., Hoganson, C., Windsor, M. W., Schenck, C. C., Parson, W. W., Migus, A., Fork, R. L., Shank, C. V.* Biochim. Biophys. Acta, **592**, 461—477 (1980).

Moscow State University

Academy of Sciences of the Estonian SSR,
Institute of Physics

Received
July 18, 1983

Valentina GODIK, A. FREIBERG, K. TIMPMANN

VALGUSENERGIA LÕKSUSTUSMEHHANISMIDE UURIMINE FOTOSÜNTEESIVATE BAKTERITE REAKTSIOONITSENTRITES PIKOSEKUNDILISE SPEKTROKRONOGRAAFIA ABIL

Purpurbakterite bakterklorofülli (BChl) fluorestsentsi eluiga (τ) mõõdeti pikosekundilise fluorestsentsspektrokronograafi abil, mida iseloomustab suur tundlikkus ja kõrge ajaline ning spektraalne lahutusvõime. Onnestus registreerida *Rhodospirillum rubrum*'i kromatofooride τ sõltuvus valgustatusest madalate (peaaegu kõik reaktsioonitsentrid (RC) avatud) ja ka küllastavate intensiivsuste piirkonnas. Tulemusena määrati ajaintervall, mis kulub valguskvandi neeldumisest BChl-s ergastuse lõksustumiseni RC-s. See aeg on 50 ± 10 ps. *R. rubrum*'i kromatofooride selektiivne ergastamine valgusega, mis neeldub põhiliselt RC-s ($\lambda \simeq 800$ nm) või antenni BChl-s ($\lambda = 370 - 380$ nm), võimaldas näidata, et valgusergastuse ülekanne ei ole limiteeritud mitte ergastuse difusiooni poolt antennis, vaid sõltub lõksustumisest RC-s. Esmane laengute eraldumise aeg *in situ* on saadud tulemuste põhjal 10—20 ps.

Валентина ГОДИК, А. ФРЕЙБЕРГ, К. ТИМПМАНН

ИЗУЧЕНИЕ МЕХАНИЗМОВ ЗАПАСАНИЯ ЭНЕРГИИ СВЕТА РЕАКЦИОННЫМИ ЦЕНТРАМИ ФОТОСИНТЕЗИРУЮЩИХ БАКТЕРИЙ МЕТОДОМ ПИКОСЕКУНДНОЙ СПЕКТРОХРОНОГРАФИИ

Времена жизни флуоресценции τ бактериохлорофилла (БХл) пурпурных бактерий измерены на пикосекундном флуоресцентном спектрохронографе. Высокая чувствительность, а также высокое временное и спектральное разрешение спектрохронографа при низкой энергии возбуждающих импульсов позволили снять световую зависимость τ для хроматофоров *Rhodospirillum rubrum* от низкой (почти все реакционные центры (РЦ) открыты) до насыщающей интенсивности. По этим данным определен временной интервал между поглощением кванта света молекулой БХл и захватом возбуждения РЦ, равный 50 ± 10 пс. Селективное возбуждение хроматофоров *R. rubrum* светом, поглощенным в значительной доле РЦ ($\lambda \simeq 800$ нм) либо БХл антенны ($\lambda = 370$—380 нм), показало, что процесс переноса энергии лимитируется захватом, а не диффузией возбуждений по антенне. Время первичного разделения зарядов *in situ* составляет, согласно полученным данным, 10—20 пс.